الجامعة الأردنيــة
كلية الدراسات العليا

التاريــــخ: / /

نموذج رقم (١٨)
اقرار والتزام بقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الماجستير والدكتوراة

أنا الطالب: أحمد علي مساعدة الرقم الجامعي: ٨٠٩٠٨٨٣
التخصص: صيدلة سريرية الكلية: الصيدلة.

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد رسائل الماجستير والدكتوراة عندما قمت شخصيا" باعداد رسالتي / اطروحتي بعنوان: ....

Impact of genetic polymorphism of ABCB1 (MDR1) 2677 G>T-A in kidney donors on tacrolimus level in Jordanian kidney transplant recipients during the early posttransplantation period.

وذلك بما ينسجم مع الأمانة العلمية المتعارف عليها في كتابة الرسائل والأطاريح العلمية. كما أنني أعلن بأن رسالتي /اطروحتي هذه غير منقولة أو مستلة من رسائل أو أطاريح أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

توقيع الطالب: .... التاريخ: ٢٩/ ٦ / ٢٠١١

The University of Jordan
Authorization Form

I, Ahmad ALi Masadeh authorize the University of Jordan to supply copies of my Thesis/Dissertation to libraries or establishments or individuals on request.

Signature:
Date: 29.06.2011

IMPACT OF GENETIC POLYMORPHISM OF *ABCB1* (*MDR1*) *2677G>T –A* IN KIDNEY DONORS ON TACROLIMUS LEVEL IN JORDANIAN KIDNEY TRANSPLANT RECIPIENTS DURING THE EARLY POST TRANSPLANTATION PERIOD

By

Ahmad A. Masadeh

Supervisor

Dr. Nailya Bulatova

Co-supervisor

Dr. Al-Motassem Yousef

This Thesis was submitted in Partial Fulfillment of the Requirements

for the Master's Degree of Clinical Pharmacy

Faculty of Graduate Studies

The University of Jordan

June, 2011

Committee Decision

This Thesis/Dissertation (Impact of Genetic Polymorphism of *ABCB1 (MDR1) 2677G>T– A* in Kidney Donors on Tacrolimus Level in Jordanian Kidney Transplant Recipients during the Early Post Transplantation Period) was successfully defended and approved on 27th June, 2011.

Examination Committee	**Signature**
Dr. Nailya R. Boulatova, (Supervisor) Assoc. Prof. of Pharmacology and therapeutics	------------------------
Dr. Al-Motassem Y. Fahmi, (Co-Supervisor) Assoc. Prof. of Pharmacology and therapeutics	------------------------
Dr. Yaser K. Bustanji. (Member) Assoc. Prof. of clinical Biochemistry	------------------------
Dr. Mayyada M. Al-Wazaify (Member) Assist. Prof. of Pharmacy Practice	------------------------
Dr. Karem H. Al-Zoubi (Member) Assoc. Prof. of Pharmacology and therapeutics (Jordan University of Science and Technology)	------------------------

Dedication

To my parents, my brothers and sisters, the Royal Medical Services staff. Thank you for all guidance and support that you have always given me, helping me to succeed. Thank you for everything.

Acknowledgments

From the early stages to the final draft of this thesis, I owe a great debt of gratitude to my supervisor, Dr. Nailya Bulatova, and co-supervisor Dr. Al-Motassem Yousef. Their advice and careful guidance were invaluable.

I would also like to thank renal transplant patients and donors who agreed to participate in the study. I am grateful to Thesis Defense Committee members for their efforts in reading and discussing this thesis.

List of Contents

List of Figures

List of Tables

List of abbreviations

Symbol or Abbreviation	Definition
ABCB1	ATP-binding cassette sub-family B member 1
AP-1	Activated Protein-1
ADR	Adverse drug reactions
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
bp	Base Pair
Co	Trough Concentration
CNIs	Calcineurin inhibitors
CyP	Cyclophilin
CYP3A	Cytochrome P450 3A
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamintetraehylacetic acid
ELISA	Enzyme-Linked Immmunosorbent Assay
EMIT	Enzyme Multiplied Immunoassay Technique
ESRD	End Stage Renal Disease
FDA	Food and Drug Administration
FK506	Tacrolimus
FKBP12	FK506 binding protein
G	Guanine Nucleotide
IL-2	Interleukin-2
MEIA	Microparticle Enzyme-Linked Immunoassay
MDR1	multidrug resistance-1
mRNA	Messenger Ribonucleic acid
NFAT	Nuclear factor of activated T cells
P	Probability value
PCR	Polymerase Chain Reaction
p-gp	p-glycoprotein
RFLP	Restriction Fragment Length Polymorphism
RMS	Royal Medical Services
RNA	Ribonucleic acid
SNP	Single Nucleotide Polymorphism
T	Thymine Nucleotide
TAE	Tris-Acetate-EDTA Buffer
TBE	Tris-Borate-EDTA Buffer
TDM	Therapeutic drug monitoring
TE	Tris-EDTA buffer
31DMT	31-O-demethyltacrolimus

IMPACT OF GENETIC POLYMORPHISM OF *ABCB1 (MDR1) 2677G>T –A* IN KIDNEY DONORS ON TACROLIMUS LEVEL IN JORDANIAN KIDNEY TRANSPLANT RECIPIENTS DURING THE EARLY POST TRANSPLANTATION PERIOD

By

Ahmad A. Masadeh

Supervisor

Dr. Nailya Bulatova

Co-supervisor

Dr. Al-Motassem Yousef

ABSTRACT

Tacrolimus is widely used as a primary immunosuppressive agent in patients post kidney transplantations. It is characterized by narrow therapeutic index and remarkable intra- and inter-individual variability, so close monitoring and achieving therapeutic trough levels of tacrolimus are essential to achieve the optimal immunosuppressive effect and to limit its toxicity during the early unstable period after transplantation. Some genetic polymorphisms in transporter proteins, such as P-glycoprotein encoded by ATP-binding cassette sub-family B member 1 *(ABCB1)* genes in donors and/or

recipients appear to be important determinants of tacrolimus pharmacokinetics during stable post-transplant period. To the best of our knowledge, there are no previous studies that assessed the impact of genetic polymorphism of *ABCB1 (MDR1) 2677G>T /A* in kidney donors on tacrolimus level in Jordanian kidney transplant recipients during the early post transplantation period. The objective of this study was to determine the role of donors' *ABCB1 G2677T/A* polymorphism on tacrolimus dose requirements, trough levels and dose-adjusted trough concentrations among Jordanian renal transplant recipients during the early, unstable period post transplantation. Donors of those renal transplant recipients who were started on tacrolimus post-transplantation (n=53) were genotyped for *MDR1 G2677T/A* using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis. Tacrolimus doses (mg/kg body weight), trough concentrations (ng/ml), dose-adjusted trough concentrations (ng/ml per mg/kg body weight) were compared among patients according to donors' allelic status for *MDR1* (*G2677T/A*). Demographic features and clinical data were obtained on admission and followed for 6 months post transplantation from patients' records and interviews. The results of the current study revealed that of the 53 donors, 28 (52.8%) were carriers of *GG*, 20 (37.7%) of *GT*, and 5 (9.4%) of *TT MDR1* alleles. Trough tacrolimus concentrations in recipients of donors carrying at least one *T* mutant alleles (*2677TT* or *2677GT,* serine phenotype) did not differ significantly from trough concentration in recipients of donors carrying homozygote wild, *2677GG* genotype (alanine) during the early 6 months post renal transplantation (*P* = 0.40, 0.62, 0.42, 0.60, 0.93, 0.66 for months 1-6, respectively). There were no other significant differences between the two donor's genotype-based groups in comparison to recipient's age, weight, body mass index, albumin serum concentrations, hematocrit

levels, calcium channel blockers use, recipient's and donors' gender, gender match between the donor and the recipient, and corticosteroid dose.

Conclusion: Donor's *MDR1* gene polymorphism has no impact on trough tacrolimus concentration during the early period post-transplantation. To date, the results of studies remain controversial and many other factors must be considered to predict variability profile of trough tacrolimus levels accurately.

I. INTRODUCTION

1. Renal transplantation

Renal transplantation is the treatment of choice for most patients with end stage renal disease (ESRD) (Magee and Pascual, 2004). Murray and co-workers performed the first successful kidney transplant in 1954; it was only possible because the donor and recipient were monozygotic identical twins (Murray, *et al.,* 2001; Taylor, *et al*., 2005). Successful renal transplantation allows freedom from the lifestyle restrictions and a complication associated with dialysis and is, therefore, associated with better quality of life. In addition, over the long term, it is more cost-effective than dialysis. Thus, transplantation remains the optimal therapy for patients with end stage renal disease (Magee and Pascual, 2004).

1.1. Renal transplantation in Jordan

Renal transplantation started in Jordan at the military hospital in the year 1972. The first operation was from a cadaveric donor. At present, there are many renal transplant centers in Jordan, all in Amman, which perform almost all renal transplant from living related donors. Since the start of the renal transplant program in Jordan, nearly 90% of the living related kidney transplant were from first degree blood relatives, 4% from second degree relatives and between 5–6% were from spouses. Almost all patients were maintained on triple immunosuppressive medications: prednisone, azathioprine, and cyclosporine during the 80 s, and later on both tacrolimus and mycophenolate were introduced to the regimen. There is less than 10% mortality rate in the treatment population. This is due mainly to infection (Said, 1999).

1.2. Advance in immunosuppression

Until the 1980s, the conventional immunosuppressive therapy consisted of azathioprine and corticosteroids. Throughout years, much effort has been done to prevent graft rejection and to improve patient outcomes. This improvement essentially results from the discovery of new class of immunosuppressive drugs, calcineurin inhibitors (CNIs) (Thervet, *et al*., 2008). Cyclosporine A, a calcineurin inhibitor (CNI), was introduced in renal transplantation in early 1980s. The major advantage was a reduction in the incidence of acute rejection and improvement in 1-year graft survival. Consequently, in combination with corticosteroids and azathioprine, cyclosporine became the standard immunosuppressive agent (Cheung, *et al*., 2007).

In the mid-1990s, tacrolimus, with a strong immunosuppressive effect was developed as an alternative to cyclosporine (Berloco, *et al*., 2001; Kim, *et al.,* 2004). Several studies reported an increased graft survival in patients using tacrolimus as initial immunosuppressive treatment. Moreover, tacrolimus is associated with less hypertension and hyperlipidemia, thereby, improving the cardiovascular risk profile (Berloco, *et al*., 2001; Margreiter, 2002).

The use of the triple therapy (tacrolimus, mycophenolate mofetil and steroids) was later suggested to play a critical role in improved clinical outcomes (Borrows, *et al*., 2004; Ciancio, *et al.*, 2004). This regimen was associated with an excellent patient and graft outcomes, with low incidence of side effects and an eventual impact in long-term graft survival (Borrows, *et al*., 2004; Sun, *et al*., 2006).

1.3. Rejection

Rejection occurs when the body recognizes that the transplanted kidney is not its own and mobilizes the immune system to fight against it (Thervet, *et al*., 2008). Rejection is

the commonest cause of early and late transplant dysfunction. There is a great variation in severity of rejection episodes and the response to treatment for them (Kalble, *et al*, 2005).

Graft rejection has been categorized into three subsets (hyper-acute, acute and chronic) depending on the onset of graft destruction (Matthew H., 2004).

1.3.1. Hyper acute rejection

Hyper acute rejection is the very early graft destruction, usually within the first 48 hours. It occurs when preformed antibodies are present in the recipient's serum and are specific for donor antigens expressed on graft vascular endothelial cells (Matthew H., 2004).

1.3.2. Acute rejection

Acute rejection has an onset of five days to three months after transplantation (Matthew H., 2004). Generally acute rejection is reversible, especially if treated (Johnson and Schoder, 2005). A high dose corticosteroid is considered the first line therapy (Taberad and Dupuis, 2005), increasing doses of current immunosuppressant, or adding a more potent immunosuppressive agent to clinical protocols is alternatives to treat acute rejection episodes (Alakulppi, *et al*., 2004).

1.3.3. Chronic rejection

Chronic rejection is a progressive decline in kidney function over time and it is the most common cause of graft loss (Taberad and Dupuis, 2005). It occurs months to years after transplantation (Matthew H., 2004). It is heralded by proteinuria and hypertension, with

a simultaneous or delayed rise in serum creatinine level over months. The main differential diagnosis is chronic nephrotoxicity (Kalble, *et al,* 2005).

While most cases of acute rejection can be treated effectively, none of the currently available therapies prevents or changes the course of chronic rejection (Johnson and Schader, 2005).

1.4. Individualizing immunosuppressive drug therapy

The establishment of an individualized immunosuppressive therapy for organ transplant patients is essential to improve the graft survival (Li, *et al*., 2007). The main concern regarding the immunosuppressive drugs is their narrow therapeutic indices. Subtherapeutic blood concentrations are associated with an increased risk of acute rejection, while overdosing may increase the risk of over-immunosuppression, with subsequent increased risk of toxicity, indicating that the immunosuppressant dosage needs to be individualized (Cheung, *et al*., 2007).

Pharmacogenetic and pharmacogenomic research, studying the effects of genetic polymorphisms on drug disposition and action, holds promise to produce useful clinical tools for individualizing immunosuppressive therapy (Jonge and Kuypers, 2008).

2. Calcineurin inhibitors

Calcineurin inhibitors (CNIs) are considered the mainstay of immunosuppression in renal transplantation. Cyclosporine and tacrolimus are currently the most widely used immunosuppressants for prevention of acute rejection following kidney transplantation (Kramer, *et al*. 2005). CNIs improved renal allograft survival, especially by reduction of the acute rejection rate in the first year after transplantation (Hariharan, *et al*. 2000). The experience gained from the laboratory and clinical use of cyclosporine and

tacrolimus has greatly advanced our knowledge about the nature of many aspects of immune response. However, the clinical practice still struggles with the shortcomings of these drugs: the significant inter- and intra-individual variability of their pharmacokinetics, the unpredictability of their pharmacodynamic effects, complexity of interactions with other agents in transplant recipients (Kapturczak, *et al*., 2004) as well as their early and long-term side effects such as nephrotoxicity (Sommerer, *et al.* 2002; Nankivell, *et al*. 2004).

2.1. Mechanism of action of CNIs

CNIs interfere with two distinct mechanisms of T- cell activation which are calcineurin-dependent and calcineurin-independent mechanisms (Kapturczak, *et al*., 2004).

2.1.1. Calcineurin- dependent mechanism of action

CNIs exert their cellular effects through binding to proteins called immunophilins. Tacrolimus binds to the 12 kDa FK506- binding protein (FKBP-12), the predominant tacrolimus binding immunophilin. The binding of tacrolimus to its respective immunophilin enhances the immunophilin's affinity to calcineurin. Formation of such a complex results in its binding to and inhibition of calcineurin. In the process of T-cell activation; calcineurin, which is a calmodulin-activated serine phosphatase, associates with and dephosphorylates inactive nuclear factor of activated T cells (NFAT). This leads to NFAT translocation to the nucleus and, in association with other transcription factors like activator-protein (AP-1), initiation of downstream events involved in T-cell activation. NFAT participates in transcriptional activation of interleukin-2 (IL-2), IL-4, and CD40L. The CNI-immunophilin complexes inhibit calcineurin activity, and, hence, prevent nuclear translocation of NFAT and cytokine gene transcription. The net result is

that CNIs block the production of cytokines such as IL-2 and inhibit T cell activation and proliferation (Taylor, *et al*., 2005); (Figure 1).

2.1.2. Calcineurin-independent mechanism of action

Both cyclosporine and tacrolimus have been noted to suppress the immune response in calcineurin-independent manner. Indeed, cyclosporine and tacrolimus interfere not only in the calcineurin/NFAT pathways but have been shown to block both the Jun N terminal kinase and p38 signaling pathways. These pathways are necessary for activation of AP-1 among other transcription factors. The interference with two distinct mechanisms of T cell activation contributes to the high specificity of immunosuppressive properties of calcineurin inhibitors (Kapturczak, *et al*., 2004).

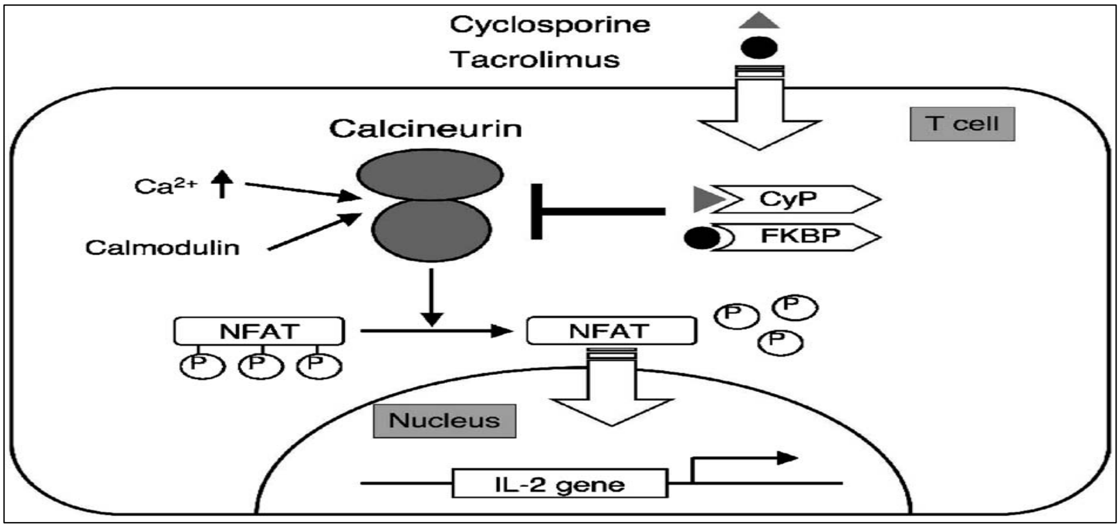


Figure 1: A schematic model of cyclosporine and tacrolimus mode of immunosuppressive action, CyP, cyclophilin; FKBP, FK506-binding protein; NFAT, nuclear factor of activated T cells; IL, interleukin **(Yano, 2008).**

2.2 . Tacrolimus

In the early 1980s scientists at Fujisawa Pharmaceuticals began testing fermented *Streptomyces* broths for their specific inhibitory properties on mixed lymphocyte cultures. The screening resulted in discovery of a soil fungus named *Streptomyces*

tsukubaensis in 1984, which produced a potent immunosuppressant given a code FK506 and later named tacrolimus (Figure 2). Initially, tacrolimus was evaluated in liver transplantation, and the FDA approved it for the prevention of liver transplant rejection in 1994 (Kapturczak, *et al*., 2004). Subsequently, its use was expanded onto transplantation of other organs such as heart, small bowel, pancreas, bone marrow, lung and kidney (Staatz and Tett, 2004), as well as treatment of atopic dermatitis as a topical formulation (Kapturczak, *et al*., 2004).

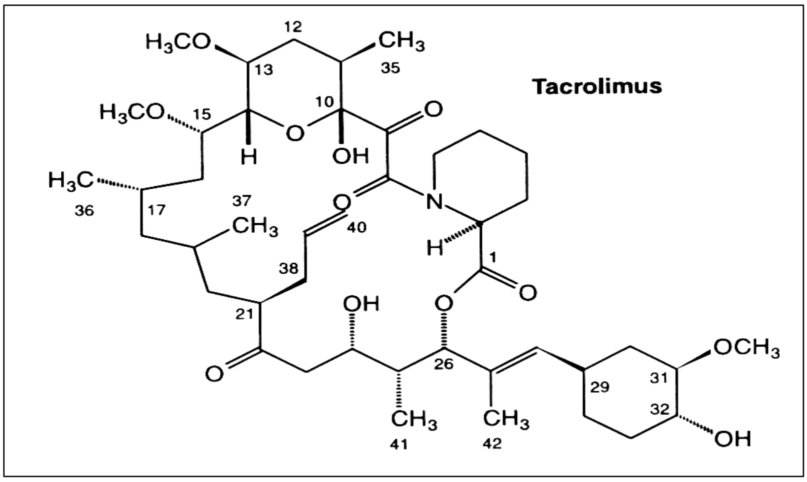


Figure 2: Chemical structure of tacrolimus (Kapturczak, *et al.*, 2004).

2.2.1. Pharmacokinetics

Tacrolimus demonstrates considerable inter- and intra-individual variability in its pharmacokinetics. Some information is now available on the factors that affect the pharmacokinetics of this immunosuppressant (Staatz and Tett, 2004).

2.2.1.1. Absorption

Large variability in the rate of absorption and absolute bioavailability of orally administered tacrolimus has been reported (Staatz and Tett, 2004). Bioavailability is poor (mean approximately 25%, but can range from 5% to 93%). Reduced

bioavailability has been reported in patients awaiting renal transplantation, in diabetic patients and following administration of food with a moderate fat content (Williams, *et al.*, 1996). In most patients tacrolimus is absorbed rapidly with peak plasma/blood concentrations obtained in 0.5-1 hour. The poor aqueous solubility of tacrolimus and alterations in gut motility in transplant patients may be partially responsible for poor and erratic drug uptake (Staatz and Tett, 2004).

Pre-systemic metabolism of tacrolimus by gastrointestinal cytochrome P450 (CYP) 3A iso-enzymes and removal by P-glycoprotein transport is extensive. P-glycoprotein lowers the intracellular concentration of tacrolimus by pumping absorbed drug back out into the intestinal lumen, it may also regulate access of tacrolimus to CYP3A enzymes and prevents these enzymes from being overwhelmed by high drug concentrations in the intestine (Williams, *et al.*, 1996).

2.2.1.2. Distribution

Following intestinal absorption, tacrolimus redistributes in blood primarily to erythrocytes. The whole-blood concentrations are, therefore, 10 to 30 times higher than those of plasma (Kapturczak, *et al.*, 2004). The uptake of tacrolimus by erythrocytes is concentration dependent, resulting in lower blood: plasma ratios at higher concentrations (Staatz and Tett, 2004). Approximately 99% of tacrolimus in plasma is bound; it is principally associated with α1-acid glycoprotein, lipoproteins, globulins and albumin. Partitioning of tacrolimus between erythrocytes and plasma is dependent on the hematocrit, tacrolimus concentration, temperature of the sample and plasma protein concentration (Williams, *et al.*, 1996). Tacrolimus is distributed extensively in the body, as evidenced by a volume of distribution as estimated from plasma data to be more than

20L/kg, indicating that the majority of the drug is outside the blood compartment, i.e. in the tissue (Kapturczak, *et al*., 2004).

2.2.1.3. Metabolism

Tacrolimus is mainly metabolized by CYP3A iso-enzymes in the liver and intestinal wall. Expression of these enzymes varies widely. The CYP3A subfamily consists of at least four isoforms: CYP3A4, CYP3A5, CYP3A7 and CYP3A43; as these isoforms have overlapping substrate specificity; it is difficult to segregate their relative contributions to the metabolism of tacrolimus (Staatz and Tett, 2004). CYP3A4 accounts for approximately 30–40% of the total CYP content in the human liver and small intestine and its expression is highly variable between individuals (Williams, *et al*., 1996). CYP3A5 is generally expressed at a much lower level than CYP3A4 in the liver but appears to be the main CYP3A isoform in the stomach and esophagus, its expression is polymorphic, with individuals exhibiting a relatively high or low level of expression (Staatz and Tett, 2004). CYP3A7 is expressed at high levels in fetal liver. More recently, c-DNA clones for a fourth CYP3A member, CYP3A43, have been isolated from the liver (Williams, *et al*., 1996).

Little is known about the substrate specificity of tacrolimus (Staatz and Tett, 2004). The exact number of metabolites is not known, the main pathways include demethylation and hydroxylation with main metabolite being 31-*O*-demethyl tacrolimus, which also possesses immunosuppressive activity (Kapturczak, *et al*., 2004).

2.2.1.4. Clearance

Low albumin or hematocrit levels in the early post-transplant period result in a higher unbound fraction and increased clearance of tacrolimus. In kidney transplant patients,

total body clearance is estimated to be around 6.8 L/h during the immediate post-transplant period. Changes in clearance later after transplantation have been shown to correlate with hematocrit and albumin levels, and the corticosteroid dose post-transplant (Kapturczak, *et al*., 2004; Dai, *et al*., 2006). Renal excretion accounts for slightly more than 2% of administered dose with less than 1% contribution of the parent drug (Kapturczak, *et al*., 2004).

Pharmacokinetic characteristics of tacrolimus in renal transplant recipients are shown in Table 1.

Table 1: Tacrolimus pharmacokinetic parameters in kidney transplant recipients (Venkataramanan, *et al*., 1995).

Parameter	Tacrolimus
Absorption half life (h) (range)	0.06-3.5
Time to C max, t max (h) (range)	0.5-6
C max at steady state, (micro g/L) (range)	0.1-0.8
Bioavailability, F (%) (range)	4-89%
Urinary excretion of unchanged drug (%)	<1%
Elimination half life (h) (range)	4-41
Total body clearance (L/h/kg) (range)	0.6-5.4

2.2.2. Non-genetic factors affecting the pharmacokinetics of tacrolimus

2.2.2.1. Age

Numerous studies already demonstrated that pediatric transplant recipients require two to four fold higher tacrolimus doses than adults to maintain similar trough concentrations. The higher tacrolimus doses required in pediatric patients have been attributed to differences in cytochrome P450 3A although differences in bowel length, hepatic blood flow and P-glycoprotein expression also need to be considered (Williams, *et al*., 1996). It is unclear whether intestinal CYP3A expression changes in parallel with

hepatic CYP3A expression during human maturation. It is not known whether P-glycoprotein expression changes with age (Staatz and Tett, 2004).

2.2.2.2 Race

African-American transplant patients require higher tacrolimus dosages (mg/kg) than Asians (Chinese or Japanese) and Caucasians. Moreover, bioavailability was significantly reduced among black patients (9.9% versus 19%). The differences between ethnic groups may result from racial differences in intestinal CYP3A or P-glycoprotein activity (Williams, *et al*., 1996).

2.2.2.3. Hematocrit and albumin concentrations

Hematocrit and albumin concentrations are generally lower in kidney transplant patients immediately post-surgery and increase significantly as the patient recovers. Since tacrolimus is strongly bound to red blood cells and serum albumin, low hematocrit and albumin concentration will result in a reduction in total drug concentration in whole blood. In such a situation, whole blood drug clearance appears to increase, while unbound drug clearance should remain the same (Staatz and Tett, 2004). Increasing in hematocrit and albumin concentrations later after transplantation will change clearance of tacrolimus and, therefore, during maintenance therapy, lower doses of tacrolimus are required to achieve similar target blood levels (Kapturczak, *et al*., 2004).

2.2.2.4. Time after transplantation

Adult patients who underwent organ transplantation have a decrease in the dosage of tacrolimus required to maintain similar trough concentrations with increasing time post-transplant. A generally considered cause for the decrease in the tacrolimus dosage is a decrease in the tacrolimus clearance with time (Williams, *et al*., 1996).

2.2.2.5. Corticosteroid dosage

The concomitant use of corticosteroids, which may induce CYP3A iso-enzymes, also has the potential to influence the tacrolimus elimination (Williams, *et al*., 1996). In a study of 303 renal transplant recipients, there was a significant correlation between the mean oral corticosteroid dosage and the tacrolimus clearance during month's 2-12 post-transplant which indicates that corticosteroids increase the tacrolimus metabolism (Undre, *et al*., 1999). Another study of liver transplant recipients demonstrated an opposite effect (Jain, *et al*., 1993).

2.2.2.6. Patient population

A higher tacrolimus clearance was found for adult renal transplant recipients compared to liver transplant recipients and healthy volunteers. The presence of low hematocrit and albumin concentrations and differences in corticosteroid dosage may be partly responsible for this observation (Chou, *et al*., 2001).

2.2.2.7. Hepatic dysfunction

Poor liver function can decrease tacrolimus clearance up to 67% and increase the elimination half-life threefold (Jain, *et al*., 1993). However, tacrolimus clearance has been reported to be similar between healthy volunteers and patients with mild hepatic impairment (Bekersky, *et al*., 2001).

2.2.2.8. Administration of food

The effect of food on the oral absorption of tacrolimus appears to be dependent on its fat content and relative time of administration. Preliminary research indicated that co-administration of low-fat food had minimal effect on the extent of absorption, but

delayed the time to reach C-max (Staatz and Tett, 2004). The study of effect of meal-timing on the tacrolimus absorption found that in the fasting state tacrolimus had a significantly greater relative bioavailability than all other treatments (Bekersky, *et al*., 2001).

2.2.2.9. Drug interactions

After oral administration, there are several factors involved in absorption of the drug which all can be the target of drug interactions: delivery to the intestine (pH, gastric emptying and food), absorption from the intestinal lumen (dissolution and lipophilicity), intestinal metabolism, active intestinal drug efflux pumps, and subsequent hepatic first pass extraction (Williams, *et al*., 1996). The interaction of the tacrolimus with many drugs commonly used in kidney transplant patients demands constant attention due to narrow therapeutic index of this drug. Since tacrolimus is a substrate for cytochrome P450 enzymes and P-glycoprotein (P-gp), it is anticipated that drugs that inhibit or induce cytochrome P450 or P-gp will affect its level (Venkataramanan, *et al.,* 1995).

2.2.3. Adverse drug reactions

Adverse reactions of tacrolimus tend to occur most frequently in the first few months after transplantation and decrease with time possibly in line with reductions in tacrolimus concentration (Williams, *et al*., 1996, Pirsch, *et al*., 1997); (Table 2). Bulatova, *et al.* (2011) have recently studied adverse effects in Jordanian renal transplant recipients who received tacrolimus-based immunosuppression and found high prevalence of hypertension (83%), hyperlipidemia (53%), anemia (51.5%), neurologic toxicity (45%), and post-transplant diabetes mellitus (27%).

Table 2: Tacrolimus adverse reactions (modified from Vicari-Christensen, *et al*., 2009)

System	Adverse reaction	Frequency, %
Cardiovascular	Hypertension	38-89
Electrolytes / Metabolic	Hyperglycemia Hyperkalemia Hypokalemia Hypophosphatemia Hypomagnesaemia Hyperlipidemia	22-70 8-45 13-29 49 16-48 31
Gastrointestinal	Diarrhea Nausea Anorexia Constipation Vomiting Dyspepsia Abdominal pain	37-72 32-46 34 23-35 14-29 28 29-59
Hematologic	Anemia Leukocytosis Thrombocytopenia Leukopenia	5-65 32 24 48
Neurologic	Headache Tremor Insomnia Paresthesia Dizziness	37-64 15-56 32-64 17-40 19
Renal	Nephrotoxic effects Urinary tract infections	52 16-34
Respiratory	Pleural effusion Dyspnea	30-36 5-29

2.2.4. Therapeutic drug monitoring and dosage

Considering the variability of the pharmacokinetic properties of tacrolimus among individuals and its narrow therapeutic index, drug monitoring is necessary to ensure appropriate immunosuppression and to avoid toxic effects (Scott, *et al*., 2003). Achieving therapeutic trough levels is of critical importance, especially during the initial period after transplantation, which is characterized by the highest risk of organ rejection. Practically, tacrolimus doses are adjusted according to the whole-blood trough

concentrations measured 12 h post-dose, just before the next dose (*Co*) (Venkataramanan, *et al*., 1995).

The frequency of blood level monitoring should be based on clinical needs. As tacrolimus is a low clearance drug, it needs several days before adjustments to the drug dosage regimen are reflected in blood levels. Blood trough levels should be monitored approximately twice weekly during the early post-transplant period and then periodically during maintenance therapy (Taylor, *et al*., 2005). Enzyme-linked immunosorbent assay (ELISA), micro particle enzyme-linked immunoassay method (MEIA) and enzyme multiplied immunoassay technique (EMIT) are used clinically for monitoring tacrolimus (Venkataramanan, *et al.,* 1995).

Dosage and target concentration recommendations for tacrolimus vary from center to another, and large pharmacokinetic variability makes it difficult to predict what concentration will be achieved with a particular dose or dosage change (Staatz and Tett, 2004). In the selection of the best immunosuppressive protocol, individual drug related toxicity, recipient-related risk factors and donor organ characteristics need to be taken into consideration (Venkataramanan, *et al.,* 1995). Current tacrolimus dosage recommendations for typical starting dose in kidney transplant is 0.15–0.3 mg/kg/day in two divided doses, 12-hourly orally (Staatz and Tett, 2004).

Therapeutic ranges have not been based on statistical approaches. Therapeutic ranges of tacrolimus after kidney transplantation are reported as a range for various times after transplant: 0-1 month, 15-20 μg/L; 1-3 months, 10-15 μg/L; and more than 3 months, 5-12 μg/L (Staatz and Tett, 2004). A recent comprehensive review by a European working group in solid organ transplantation provides evidence for target levels in different types of transplantation (Wallemacq, *et al*., 2009).

Protocols from manufacturing company "Hikma" of recommended blood levels of tacrolimus and guide for adjustment during the early period post transplantation are presented in Tables 3 and 4.

Table 3: Recommended blood levels of tacrolimus (Pocket guideline of Prograf® recommendations from Hikma, manufacturing company of Prograf® (Tacrolimus) in Jordan**)**

Blood level (ng/ml)	Time post-transplantation
15 – 20	0 - 14 days
10 – 15	15 - 28 days
7- 10	Week 4 – month 6

Table 4: Tacrolimus dose adjustment during dose titration (Pocket guideline of Prograf® recommendations from Hikma, manufacturing company of Prograf® (Tacrolimus) in Jordan**)**

Action	Blood level (ng/ml)
Double the dose	< 5
Increase the dose by 20% to 40%	5 – 10
Reduce the dose by 20% - 40%	20 – 30
Reduce the dose by 50% or more	30 – 40
Skip the evening dose and on the next day, reduce the dose by 50%	> 40

3. ABCB1 (P-GLYCOPROTEIN/MDR1)

P-glycoprotein (Pgp) is a large trans-membrane protein of 170 kD that functions as an energy-dependent pump transporting a variety of compounds extracellularly. It belongs to the large ATP binding cassette transporter family and is defined as ABCB1. Pgp is composed of 1280 amino acids forming two analogous halves with 43% sequence homology. Two domains interact to form a functional transporter; each part is

composed of six transmembrane α-helices and a nucleotide binding domain (NBD) (Figure 3).

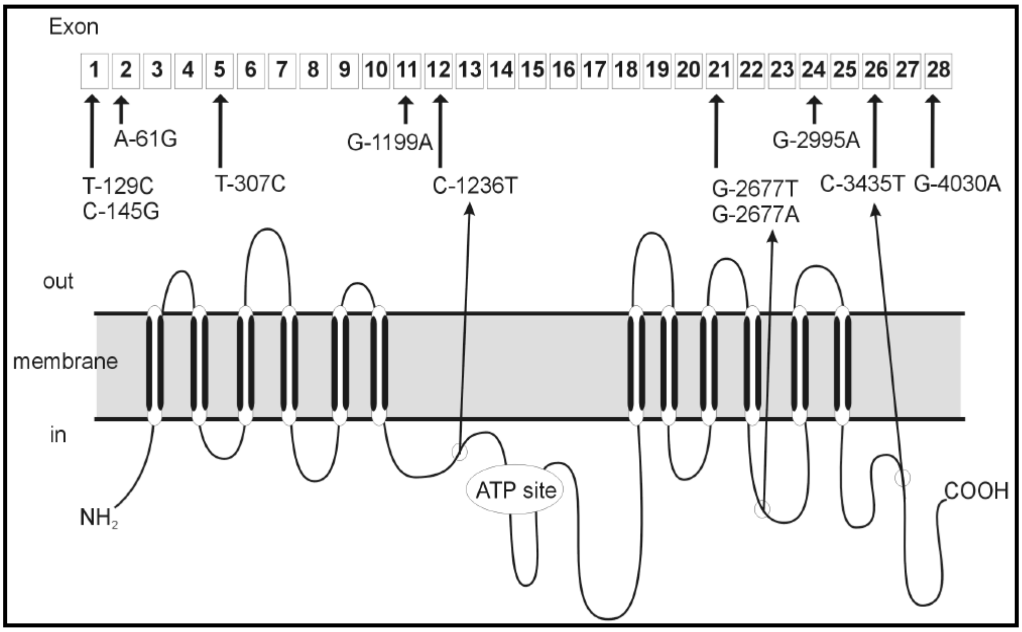


Figure 3: Schematically depicted primary structure of P-glycoprotein. Arrows represent known *SNPs* and their relation to the structure of the protein **(Pechandova, *el al*., 2006).**

NBD is a highly conservative area within the ABC transporter family that is involved in binding of ATP and hydrolysis of ATP to release energy, which is utilized for active uphill transport. The shape of Pgp reminds a cylinder with a maximal height of 8 nm and 10 nm in diameter (Higgins, *et al*., 1997; Rosenberg, *et al.,* 1997).

P-gp is widely expressed in tumor cells, but also on the apical surface of epithelial cells of the intestine, biliary canalicular membrane of hepatocytes, on the luminal surface of the capillary cells forming the blood brain barrier, in brush border membranes of proximal tubules in the kidney, in the adrenal cortex and in placenta (Kerb, *et al*., 2001; Sakaeda, *et al*., 2002; Leonard, *et al.,* 2003). P-gp serves as a barrier against entry of compounds into the body, as well as from entering tissues (Figure 4).

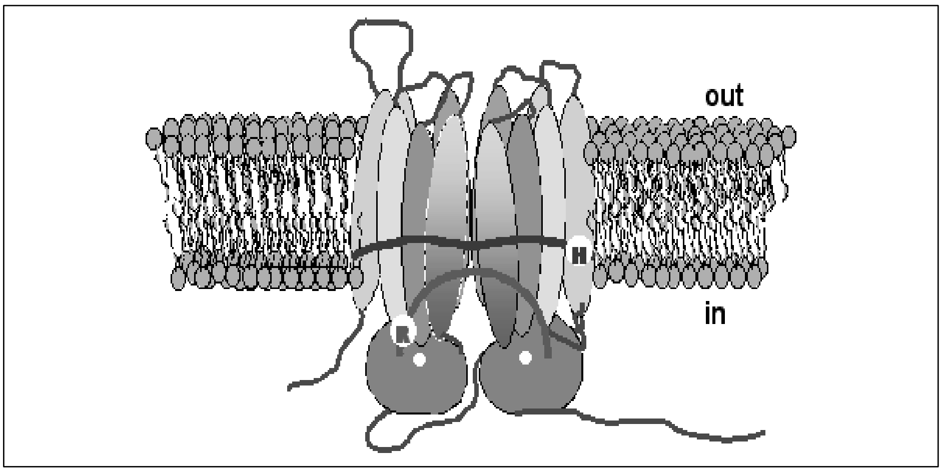


Figure 4: Three-dimensional presentation of the P-glycoprotein efflux pump in a bilayer membrane (Brinkmann and Eichelbaun, 2001).

The substrate specificity of the P-glycoprotein is extremely wide. List of P-gp substrates, inducers, and inhibitors is provided in Table 5. P-gp participates in removal of drugs from the organism. Because of that, genetic variations that alter protein function or expression of P-gp can substantially affect intestinal absorption, elimination, and penetration of drugs into brain, germ cells, and the fetus (Kotrych, *et al.*, 2007).

Table 5: Substrates, inducers and inhibitors of MDR1 (modified from Zhou, 2008)

Substrates	Inducers	Inhibitors
Anticancer agents	Amiodarone	Amiodarone
Daunorubicin	Bromocriptine	Astemizole
Docetaxel	Chlorambucil	Atorvastatin
Etoposide	Cisplatin	Bromocriptine
Irinotecan	Clotrimazole	Carvedilol
Mitoxantrone	Colchicine	Chlorpromazine
Paclitaxel	Cyclosporine	Clarithriomycin
Vincristine	Daunorubicin	Cyclosporine
Antihypertensive agents	Dexamethasone	Diltiazem
Diltiazem	Diltiazem	Dipyidamole
Losartan	Doxorubicin	Erythromycin
Anti-arrhythmics	Erythromycin	Felodipine
Digoxin	Etoposide	Fluoxetine
Quinidine	Flurouracil	Itraconazole
Verapamil	Hydroxyurea	Ketoconazole
Antimicrobial agents	Insulin	Midazolam
Doxycycline	Methotrexate	Paroxetine
Erythromycin	Midazolam	Progesterone

Itraconazole Ketoconazole Levofloxacin Rifampin Tetracycline **Anticonvulsants** Phenobarbital Phenytoin **H2-antagonists** Cimetidine Ranitidine **Immunosuppressants** Cyclosporine Sirolimus Tacrolimus **Steroid hormones** Aldosterone Dexamethasone Methylprednisolone	Mitoxantrone Nifedipine Phenobarbital Phenytoin Reserpine Rifampicin St John's wart Tacrolimus Tamoxifen Verapamil Vinblastine Vincristine	Propafenone Quinidine Quinine Reserpine Ritonavir Sertraline Tacrolimus Tamoxifen Verapamil Vinblastine

4. Pharmacogenetics and pharmacogenomics

Both terms are synonymous for all practical purposes; pharmacogenetics assesses the relationship between polymorphisms of a single gene and the pharmacokinetic and pharmacodynamic behavior of a drug, whereas in pharmacogenomics more complex models of genetic variability are used to analyze how the individual's genomic composition as a whole affects drug behavior. The latter approach assesses the contribution of multiple genes, as well as gene-to-gene interactions, and is theoretically the most promising approach (Jonge and Kuypers, 2008; Mourad, *et al.,* 2008). Evolving areas of interest include how polymorphisms in genes that encode metabolizing enzymes, drug transporters, and drug targets can be used to predict inter-individual variations in drug response and how this information can be used to individualize therapy. In spite of advances in the sciences of pharmacokinetics and pharmacodynamics toward individualization of patient drug therapy, most of drug therapy today is still done by protocol that does not consider individual patient variations (Wavamunno and Chapman, 2008).

4.1. Genetic polymorphism

Genetic polymorphism is the occurrence together in the same population of more than one allele or genetic marker at the same locus with the least frequent allele or marker occurring more frequently than can be accounted for by mutation alone (Mathew, 2010). On the other hand, single nucleotide polymorphism (SNP) can be defined as DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered. Each individual has many single nucleotide polymorphisms that together create a unique DNA pattern for that person (Daly, 2010).

On the basis of their location in the genome, SNPs can be classified as random SNPs (most common SNPs found in intergenic deoxyribonucleic acid region or in the introns of genes), coding SNPs (found in the exons representing the translated regions of a gene), and non-coding SNPs (found outside the translated regions of a gene such as the promoter and 5' and 3' un-translated regions), Of specific interest to pharmacogenetics are coding SNPs because they can result in encoded amino acid changes. Non-synonymous SNPs refer to polymorphisms that result in amino acid changes, whereas synonymous SNPs do not lead to amino acid changes (Marzolini, *et al*., 2004).

4.2. *MDR1 G2677T/A* SNP

The *MDR1* gene, which encodes P-gp, is located on the long arm of chromosome 7 and consists of 28 exons (Balarm, *et al.,* 2003). More than fifty SNPs have been reported in the *MDR1* gene (Ishikawa, *et al*., 2004); (Table 6).

The first genetic polymorphism identified in the *MDR1* gene was *G2677T* (Mickley, *et al*., 1998). This SNP at exon 21 in position 2677 is of particular interest because it has significant pharmacological effects and is associated with alteration of P-gp expression

and/or function (Eichelbaum, *et al.,* 2004). It can result in 2 distinct amino acid changes, namely, *Ala893Ser* (*G2677T*) and *Ala893Thr* (*G2677A*); (Hoffmeyer, *et al*., 2000). Impact of the *G2677A/T* mutation on ribosome stalling could be caused by a non-synonymous polymorphism. Biochemical analysis has confirmed that mutation from alanine to serine or threonine may alter drug transport (Sakurai, *et al.,* 2007). The evidence suggests that this mutation likely affects drug-induced *ATP*-ase activity (Fung and Gottesman, 2009).

Table 6: Summary of Genetic Polymorphisms in *MDR1* (Toshiyuki S, *et al*., 2002).

Position	**Location**	**Effect**
A1a/-41G	Intron	Noncoding
C-145G	Exon 1a	Noncoding
.T-129C (T12C)	Exon 1b	Noncoding
C-4T	Exon 2	Noncoding
G-1A	Exon 2	Noncoding
A61G	Exon 2	Asn21Asp
G5/-25T	Intron	
G5/-35C	Intron	
T307C	Exon 5	Phe103Leu
C6/+139T	Intron	
A548G	Exon 7	Asn183Ser
G1199A	Exon 11	Ser400Asn
C1236T	Exon 12	Silent
C12/+44T	Intron	
C1474T	Exon 13	Arg492Cys
T17/-76A	Intron	
A17/+137G	Intron	
C2650T	Exon 21	Silent
G2677(A,T)	Exon 21	Ala893Thr (G2677A) Ala893Ser (G2677T)
A2956G	Exon 24	Met986Val
G2995A	Exon 24	Ala999Thr
A3320C	Exon 26	Gln1107Pro
C3396T	Exon 26	Silent
T3421A	Exon 26	Ser1141Thr
C3435T	Exon 26	Silent
G4030C	Exon 28	Silent
A4036G	Exon 28	Silent

The allelic frequency of *MDR1* SNPs varies widely among different ethnic groups (Hoffmeyer, *et al.* 2000). In general, *MDR1* SNPs are found in all studied populations (Cavaco, *et al.* 2003; Kaya, *et al.* 2005; Pechandova, *et al.,* 2006), and the chance of carrying a particular mutant allele is higher in some populations (Zhou, 2008). African-Americans have relatively low *G2677T* allelic frequency (10%) than other populations, while Caucasian (42–46%), Mexican-American (40%), and Asian-American (45%) are more likely to carry this mutant allele (Chinn and Kroetz, 2007). Overall, the presence of the corresponding wild-type alleles is more frequent than that of mutant alleles (Schaeffeler, *et al.,* 2001).

4.3. *MDR1* Polymorphisms and Tacrolimus pharmacokinetics

The association of SNPs with protein expression or changes in kinetics of *MDR1* substrates indicates that it is a major determinant in the absorption, distribution, and elimination of drugs (Eichelbaum, *et al*., 2004). Studies have shown that there is a significant association of the donor's *MDR1*gene polymorphism with the trough level of tacrolimus in liver transplant recipients during the first 2 weeks after surgery (Hosohata, *et al*., 2009), and the recipient's *MDR1*gene polymorphism with the trough level of tacrolimus in renal transplant patients on day 28 after transplant (Tsuchiya, *et al.*, 2004). The most important relation was noted for the exon 21 *2677G (T/A)* SNP. The tacrolimus dose requirement was 40% higher and the concentration/dose ratio was 36% lower in homozygous mutant than wild-type carriers in renal transplant recipients at 1 month post transplantation (Anglicheau, *et al*., 2003). On the other hand, there was no association between 10 SNPs of the *MDR1* gene and the tacrolimus concentration/dose ratio during the first postoperative days after liver transplantation (Goto, *et al*., 2004).

Table 7 summarizes studies regarding relation between *MDR1 G2677T/A* polymorphisms and tacrolimus pharmacokinetics.

Understanding the pharmacogenetics of tacrolimus may enable individualized therapeutic dosing, resulting in adequate immunosuppression with minimization of adverse reactions, and, ultimately, may result in greater allograft survival (Vicari-Christensen *et al.,* 2009).

Table 7: Summary of studies concerning relation between *MDR1 G2677T/A* polymorphisms and tacrolimus pharmacokinetics

Study	Population studied	Effect
Studies that demonstrated lack of *MDR1 G2677T/A* polymorphisms impact on tacrolimus pharmacokinetics		
Zheng, *et al*., 2003	14 Caucasian and 3 African American pediatric heart transplant recipients.	No significant differences in tacrolimus blood level per dose/ kg/ day between recipient's *MDR1 G2677T* and *C3435T* genotype at 3 months, but both were found to have a significant association with tacrolimus blood level per dose/ kg/ day at 6 and 12 months, recipients with *CC* and *GG* required a higher dosage of tacrolimus to achieve similar blood levels compared with *CT/TT* or *GT/TT* recipients.
Studies that demonstrated impact of *MDR1 G2677T/A* polymorphisms on tacrolimus pharmacokinetics		
Wang, *et al*., 2005	86 Chinese adult renal transplant recipients	Recipient's *MDR1 G2677T/A, C3435T* polymorphism are correlated with the whole blood concentration of tacrolimus. To obtain the similar blood concentration, the recipients with *GG* and *CC* should take the drug at a higher dose than those with *CT* and *TT* in renal transplant patients at three, six, and twelve months post transplantation.
Roy, *et al*., 2006	44 adult Caucasian renal transplant recipients.	The complete absence of *CYP3A5*3* allele and the accumulation of less than three copies of *MDR-1* (*T-129C, C3435T* and *G2677T*) polymorphisms in renal transplant recipients are associated with lower tacrolimus blood levels identifying these genotypes as markers for patients requiring higher tacrolimus doses during the first week post transplantation.
Elens, *et al*., 2007	150 adult liver recipients and corresponding donors.	Donors' *ABCB1* genetic polymorphisms significantly influenced tacrolimus hepatic concentrations of recipients, whereas the impact on blood concentrations seemed negligible. Among these *ABCB1* polymorphisms, the donor's *1199G>A,* and *2677G>T/A* SNPs seemed to reduce the activity of P-gp on tacrolimus in recipients.

Mendes, *et al*., 2009	30 Caucasian renal transplant recipients.	Recipients receiving tacrolimus and heterozygous for the *MDR1 1236 CT* showed concentrations 44.4% higher than those of wild-type individuals. Recipients carrying the *MDR1* 2677*G>T, A* mutation showed tacrolimus concentration that were 44.7% higher than the wild-type individuals.
Ulemat, 2010	57 Jordanian renal transplant recipients and corresponding donors.	Trough tacrolimus concentrations in stable kidney recipients of donors carrying at least one *T* mutant alleles (*2677TT* or *2677GT*) showed 27% higher trough concentration than recipients of donors carrying homozygote wild *2677GG* genotype.

4.4. Current status of studies on impact of *MDR1* polymorphism of donors in relation to tacrolimus level in Jordanian kidney transplant recipients during the early post transplantation period

Up to date there is no study on the influence of donors *MDR1* genetic polymorphism on tacrolimus dose requirements in Jordanian kidney recipients during the early period post transplantation.

Taking together the facts of:

1. presence of *MDR1* in the kidney with proven contribution to tacrolimus excretion and having interplay with kidney metabolizing enzymes;
2. the knowledge that the expression of *MDR1* depends on genetic polymorphism in *MDR1* gene; and
3. that tacrolimus pharmacokinetics is not stable during early period post transplant,

We hypothesized that donor *MDR1* genotype may contribute to tacrolimus dose requirements in kidney recipients during that early unstable period post transplantation.

II. AIMS AND OBJECTIVES

The present study was conducted to investigate the impact of *MDR1* genetic polymorphism of donors on the pharmacokinetics of tacrolimus in Jordanian renal transplant patients during the early 6 months post transplantation.

Specifically, the following objectives were the focus of the study:

1. Estimate the mean dose, trough level and mean dose-adjusted level of tacrolimus in the study population, and compare it with the recommended doses and with the dose requirements in other population.
2. Estimate the genetic frequencies of *MDR1 G2677T/A allele* in the study population.
3. Evaluate the impact of donor *MDR1 G2677T/A allele* on tacrolimus doses, blood levels and dose adjusted concentrations.

III. METHODOLOGY

1. Materials

- Crimson Taq DNA polymerase (New England Biolab, USA).
- 50 bp DNA step ladder (New England Biolab, USA).
- dNTP (Promega Corporation, USA).
- Nuclease free water.
- Agarose; molecular biology grade.
- Tris-base (Trishydroxymethylaminomethane); molecular biology grade.
- EDTA (ethylene diamine tetraacetate disodium salt); analytical grade.
- TBE (Tris- Borate- EDTA Buffer); molecular biology grade.
- Boric acid; analytical grade (MERK, USA).
- Ethedium bromide; molecular biology grade (Promega Corporation, USA).
- Wizard Genomic® (DNA purification kit) (Promega Corporation, USA) (Cell lysis solution, Nuclei lysis solution, DNA rehydration solution, Protein precipitation solution, RNAse solution).
- Rsa1 Restriction endonuclease (New England Biolab, USA).
- Ban1 Restriction endonuclease (New England Biolab, USA).
- *MDR1 G2677T/A Primer* (The Midland Certified Reagent Company).

2. Equipment's

- PTC-100 Peltier Thermal Cycler (MJ research, USA).
- Sigma 1-15 Microcetrifuge (Sigma Corporation, USA).
- Horizontal cell (Bio-Rad, USA).
- Power supply (Bio-Rad, USA).
- UVP UV-Trans-illuminator (Alpha Imager, Alpha Innotech).
- UVP gel documentation system (multi-image TM light cabinet).

3. Ethical approval

The study was approved by the Local Research Ethics Committee of the King Hussein Medical Centre (KHMC) (Appendix I), and written informed consent (Appendix II) was obtained from all participants.

4. Patients

Consequent kidney transplant recipients treated with tacrolimus and attending regularly kidney transplant clinic at KHMC during the first six months post transplant and their corresponding donors were invited to participate in this study. All patients treated with tacrolimus used capsule formulation (Prograf®; Fujisawa, Munich, Germany). Both donors and recipients were asked to sign informed consent (Appendix II).

4.1. Inclusion criteria

Donors of kidney transplant recipients with the following characteristics:

1. Recipient with newly transplanted kidney (zero time - 6 months).
2. Recipient with current tacrolimus therapy.
3. Recipient age >18 years.
4. Regular follow up visits during the early 6 months post transplantation.

4.2. Exclusion criteria

Donors of kidney transplant recipients with at least one of the following characteristics:

1. Pregnant during study.
2. Recipient taking medications known to have clinically significant interaction with tacrolimus such as azole antimycotics (e.g., fluconazole), macrolide antibiotics (erythromycin and clarithromycin), antiepileptic (phenobarbital,

phenytoin and carbamazepine), rifampin, metoclopramide. Exception was made for calcium channel blockers (CCBs) as treatment necessary.

3. Recipient discontinuation of tacrolimus therapy.

5. Data collection

All donors participating in the study provided their demographic data. All kidney transplant recipients had clinical and laboratory assessment: patients were interviewed; tacrolimus doses as well as demographic and clinical data were obtained from medical files. All diseases that occurred post transplantation were assessed. Data collection form (Appendix III) was designed to include the following data:

- Demographic data for donors and recipients
- Transplantation and past medical history of recipients
- Current drug treatment for recipients including route, dose, frequency and duration
- Clinical laboratory data of recipients
- Tacrolimus level and recommended dose at each clinic visit for recipients
- History of rejection in recipients
- Tacrolimus neurological, dermatological and miscellaneous ADRs in recipients

6. Blood samples collection

Blood samples (approximately 3 ml) were obtained from 53 Jordanian kidney transplant donors whose recipients were treated with tacrolimus. Blood was collected in EDTA-tube for genotyping analysis and stored at 4^oC until DNA extraction.

7. Tacrolimus concentration measurements

All procedures of tacrolimus concentration measurements were performed in the clinical laboratory of the KHMC. Whole blood samples from patients were drawn in EDTA-tube during hospital stay post transplantation or during routine visits to nephrology outpatient clinic for drug concentration measurements. The concentrations were obtained directly at the laboratory. Trough concentration of tacrolimus was measured in whole blood by IMx Tacrolimus II assay which utilizes micro particle enzyme immunoassay (MEIA) in the Abbott IMx system (Tacrolimus II; Abbott Laboratories, Diagnostic Division, Abbott Park, IL. USA). Blood levels were reported for all patients, and dose-adjusted concentrations were calculated by dividing the concentration by the corresponding total daily dose on milligram per kilogram basis.

8. Renal function assessment

The function of the grafted kidney was assessed based on serum creatinine, blood urea nitrogen and creatinine clearance. The estimated creatinine clearance was calculated for each patient according to Modification of Diet in Renal Disease (MDRD formula) (Levey, *et. al*., 1999):

$$\mathbf{GFR\ (ml/min/1.73\ sq\ m) = 186.3\ (SCr)^{-1.154} \times (age,\ yr)^{-0.203} \times 0.742\ (if\ female),}$$

where age is expressed in years, SCr is the serum creatinine in mg/dL.

In normal subject, serum creatinine level is 0.5–1.5 mg/dl, and creatinine clearance is approximately 120 mL/min (Comstock, 2002).

9. Genotyping analysis

9.1. Extraction of genomic DNA from blood samples

Commercial kit (Wizard genomic DNA purification kit, Promega), was used to extract genomic DNA from the whole blood samples. According to the kit manufacturer recommendation, the genomic DNA was extracted from all blood samples (Appendix IV).

The DNA yield was confirmed by running the samples on 1 % agarose gel. In brief, 1% agarose solution was prepared in 0.5X Tris-boric-EDTA Buffer (TBE) and left to solidify at room temperature. After solidification, the gel was transferred to electrophoresis tank, which was filled with TBE buffer. After that 5 µl of DNA sample was mixed with 1 µl of 6x gel loading buffer and loaded into the well. Then the gel was run for approximately 1 hour at 130 volt and photographed by UV-documentation system.

9.2. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

9.2.1. PCR-RFLP analysis of *MDR1 G2677T/A*

PCR-Restriction Enzyme Assays was performed to detect the allele's variant in the present study. The sequences for the forward primer and reverse primer are shown in Table 8. The design of primers was made by Ms. Ola Diab (MSc Clinical Pharmacy).

Table 8: Sequences and characterization of *MDR-1 2677* allele primers

	PRIMER	TM	FROM	TO	M.WT.
F	5'TTTAGTTTGACTCACCTTCCCG'3	52.97°C	12394871	12394892	6627.9 g/mol
R	5'TGTTTTGCAGGCTATAGGTGCC'3	54.84°C	12395078	12395099	6772.6 g/mol

F, forward primer; R, reverse primer, TM, melting temperature; M.WT, molecular weight, Positions of primers are according to NT_007933.14 accession number.

9.2.2. Preparation of primers

Primers for amplification of *MDR1* region were obtained from The Midland Certified Reagent Company as dried lyophilized powder. The working concentration required for PCR was 6.25 nM for each primer. In order to obtain such concentration, forward and reverse primers were prepared as following:

– Forward primer (total amount supplied was 52.8 nmol)

1. The tube with primer was centrifuged for 15 second at 3000 x *g* to collect the primer at the bottom of the tube.
2. The powder was reconstituted with 84.48 µl Tris-EDTA Buffer (TE-buffer, Appendix IV) (pH = 8) in order to obtain a stock solution of primer with a concentration of approximately 625 nM, and was rehydrated for 2 minutes, then vortexed for 15 seconds.
3. Then 6 µl of this stock solution was diluted with 54 µl TE buffer (pH = 8) to obtain master solution with a concentration of 62.5 nM.
4. Working solution was prepared by diluting 6 µl of master solution with 54 µl of 10mM Tris buffer (pH = 8) to obtain the required concentration of 6.25 nM for PCR.
5. Reverse primer (total amount supplied was 42.2 nmol).
6. The reverse primer was prepared as described above for forward primer except that the whole amount of the primer was reconstituted in 67.5 µl of TE buffer (pH = 8).

9.2.3. Preparation of PCR mix

For preparation of PCR mix, we prepared two master mixes, master A containing (½ 5X Crimson taq reaction Buffer/750mM MgSO4, forward primer (6.25 nM), reverse

primer (6.25 nM), nuclease free water) and Master B (½ 5X Crimson taq reaction Buffer, dNTP (10mM), Crimson taq (1.25U), nuclease free water).

To prepare one PCR mix (final volume is 60 µl), the following components were collected in 0.2 ml PCR tube on ice:

1. 7 µl GoTaq reaction Buffer/750mM $MgSO_4$.
2. 1.4 µl of 6.25 nM forward primer (6.25 nM).
3. 1.4 µl of 6.25 nM reverse primer (6.25 nM).
4. 2.8 µl DNA template.
5. 1.4 µl dNTP mix, 10mM each.
6. 0.35 µl Crimson taq DNA polymerase.
7. Q.S with nuclease free water (20.65 µl).
8. 25 µl mineral oil.

After that the tube was centrifuged at 3000 x *g* for 10 seconds, and then placed in thermal cycler that has been heated to 95°C.

9.2.4. PCR conditions

PCR was started with the following program:

1. Initial denaturing at 95°C for 5 minutes
2. 39 cycles of:
   - Denaturing at 94°C for 1 minute.
   - Annealing at 58°C for 1 minute.
   - Extension at 68°C for 1 minute.
4. Final extension at 72°C for 10 minutes
5. Store at 4°C

After that the annealing step was optimized according to the result of this PCR program.

9.2.5. Digestion of PCR product with *Ban I/Rsa I*

PCR product was digested by *BanI* or *Rsa I* restriction endonucleases according to the enzyme manufacturer recommendations (New England Biolab, USA). The digestion mix was prepared for one reaction in 0.2 ml PCR tube to final volume of 15 µl on ice as following:

1. 10 µl PCR product.
2. 1.5 µl (10x) restriction buffer.
3. 2.5 µl nuclease free water.
4. 1 µl (5 units) restriction endonuclease.

After that the mixture was pipetted, centrifuged at 3000 x *g* for 10 second to collect the mix at the bottom of the tube, and placed in incubator that has been preheated to 37^{o}C. Then the PCR product was digested at 37^{o}C for one hour.

9.2.6. Gel electrophoresis of digested product

– Forming the gel (for a 2.5% gel, 115mL volume) (Sambrook and Russell, 2001):

1. 2.87g of agarose was weighed into a 250 mL conical flask.
2. 115mL of 1 X TBE was added and swirled to mix.
3. Then it was microwaved for about 1 minute to dissolve the agarose.
4. It was left to cool on the bench for 5 minutes down to about 60°C (just too hot to keep holding in bare hands).
5. The gel was poured slowly into the gel tank. Any bubbles were pushed away to the side using a disposable tip. The comb was inserted and double checked that it was correctly positioned.
6. It was left to set for at least 30 minutes.

7. 1 X TBE Buffer was poured into the gel tank to submerge the gel to 2-5mm.depth. This is the running buffer.
8. The first well was loaded with marker.
9. The samples were loaded and finished the final lane with marker.
10. Gel tank was closed, switched on the power-source, and the gel was run at 130 volt (8 volt per cm).
11. The progress was monitored by reference to the marker dye.
12. The gel tank was switched off and unplugged.
13. The gel was submerged gently in ethidium bromide solution.
14. It was left to stain for 15 minutes at room temperature.
15. The gel was de-stained by soaking in water for 5 minutes.
16. The gel was removed from de-staining solution.
17. The gel was carried to a UV light box (UV-trans-illuminator) and a picture of the fluorescent ethidium bromide-stained PCR product was taken with the Alpha Imager camera. Expected results of PCR-RFLP are shown in figures (5 and 6) below:

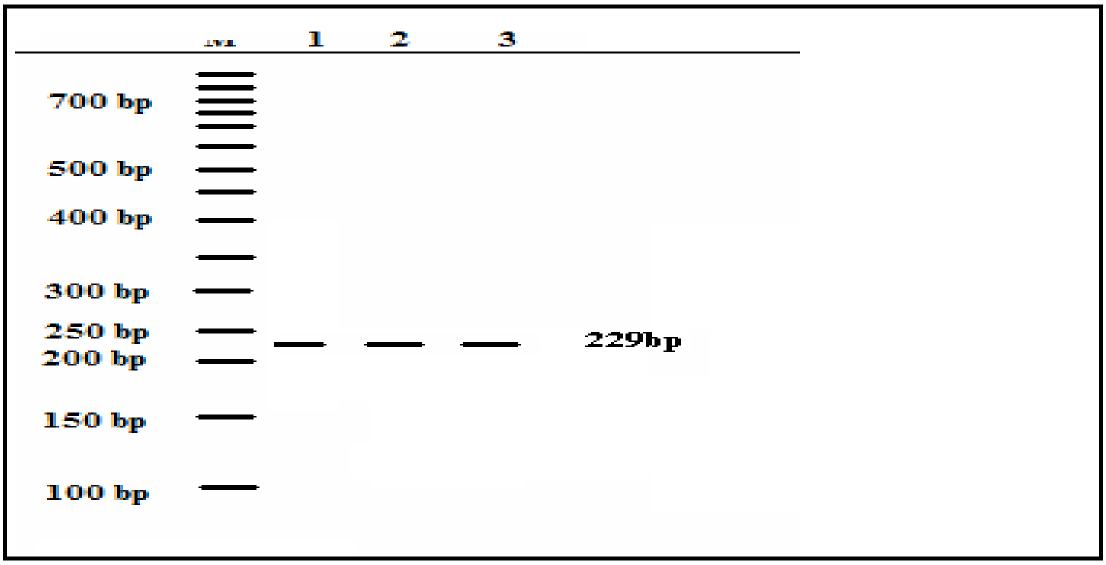


Figure 5: Schematic presentation of expected PCR result for the *ABCB-1 G2677T/A* allele. Lane M: 100 bp DNA step ladder. Lanes 1-3: PCR product (229 bp).

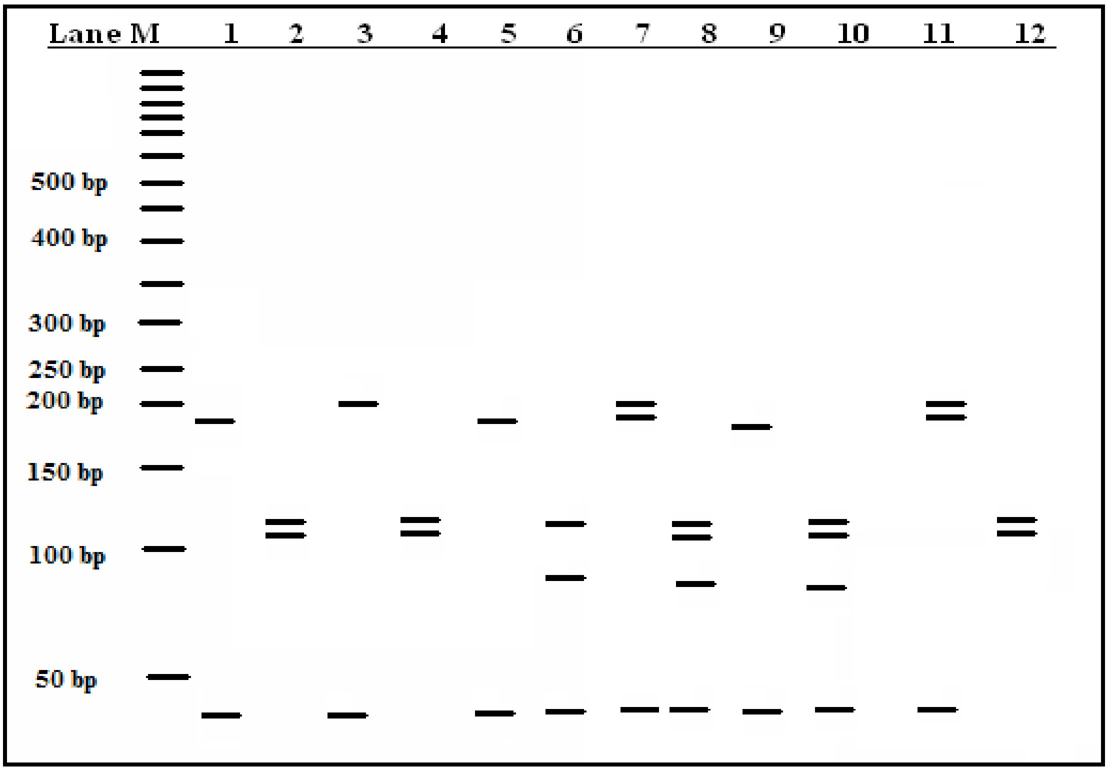


Figure 6: Expected RFLP product of *ABCB-1 G2677T/A* allele. Lane M: 50 bp DNA step ladder; Lane 1: *Ban 1* digested RFLP product (186 & 23 & 20 bp); Lane 2: *Rsa 1* digested RFLP product (123 and 106 bp); Lanes 1 & 2: Wild type *GG*; Lane 3: *Ban 1* digested RFLP product (209 & 20 bp); Lane 4: *Rsa 1* digested RFLP product (123 and 106 bp); Lanes 3 & 4: Homozygote mutant type TT; Lane 5: *Ban 1* digested RFLP product (186 & 23 & 20 bp); Lane 6: *Rsa 1* digested RFLP product (123, 82 & 24 bp); Lanes 5 & 6: Homozygote mutant type AA; Lane 7: *Ban 1* digested RFLP product (209, 187 & 22 & 20 bp); Lane 8: *Rsa 1* digested RFLP product (123 & 106 & 24 & 82 bp); Lanes 7& 8: Heterozygote mutant type TA; Lane 9: *Ban 1* digested RFLP product (186 & 20 & 23 bp); Lane 10: *Rsa 1* digested RFLP product (123 & 106 & 82 & 24 bp); Lanes 9 & 10: Heterozygote mutant type GA; Lane 11: *Ban 1* digested RFLP product (186 & 209 & 20 & 23 bp); Lane12: *Rsa 1* digested RFLP product (123 & 106 bp); Lanes 11 & 12: Heterozygote mutant type GT.

10. Estimation of genotype frequencies

Genotype frequencies for *ABCB1 G2677T/A* allele among Jordanian population were estimated from the results of PCR-RFLP test for 53 kidney transplant donors. This estimation was according to the following formulas (Brooker, 2005):

$$\textbf{Allele frequency} = \frac{\textbf{number of copies of an Allele in a population}}{\textbf{total number of all alleles for that gene in a population}}$$

$$\text{Genotype frequency} = \frac{\text{number of individuals with a particular genotype in a population}}{\text{total number of all individuals in a population}}$$

Also, genotype and allele frequency were compared with Hardy-Weinberg equilibrium and with other studies.

11. Statistical analysis

Before statistical analysis, data set was tested for normality of distribution using Kolmogorov-Smirnov Z test. All values were represented as means (± standard deviations). For statistical analysis based on different genotype groups, independent-samples t-test was used. Wild genotypes were compared against the combined group of homozygote mutant genotype and heterozygote mutant genotype.

All calculations were performed with SPSS 17.0 for windows. *P* value < 0.05 was considered as statistically significant.

IV. RESULTS

1. Demographic data

58 kidney transplant recipients and 53 corresponding kidneys donors who met the inclusion criteria participated in the study; the remaining 5 donors were not available because there was no opportunity to contact them.

Characteristics of recipients are summarized in Table 9.

Table 9: Demographic data of Jordanian kidney transplant recipients and corresponding donors.

Parameter	**N (%) or mean±SD (range)**
RECIPIENTS (n=58)	
Gender, N (%) Men	 37 (63.8%)
Age [years, mean± SD (range)]	36.29±10.39 (19-63)
Body mass index [kg/m^2, mean± SD (range)]	24.5±4.65 (14.3-36.7)
Patients who were on HD* before transplantation, N (%)	49 (84.5%)
Period of dialysis before transplantation [months, mean± SD (range)]	14.16 ± 19.18 (0-108)
Patients who hade more than one transplantation, N (%)	1 (1.7%)
Hospital stay during the last transplantation [days, mean± SD (range)]	13.46 ± 5.6 (6-28)
Primary kidney disease, N (%) Hypertensive nephropathy Other (small kidneys, solitary kidney, urine obstruction) Glomerulonephritis Unknown Chronic pyelonephritis Diabetic nephropathy Polycystic Kidney Disease	 19 (32.8%) 14 (24.1%) 8 (13.8%) 6 (10.3%) 4 (6.9%) 4 (6.9%) 3 (5.2%)

Concomitant condition, N (%)	
Hypertension	48 (82.8%)
Diabetes mellitus	8 (13.8%)
Hyperlipidemia	5 (8.6%)
CAD**	3 (5.2%)
SLE***	3 (2.4%)
Hepatitis C	1 (0.8%)
Post transplant conditions (Tacrolimus-induced), N (%)	
Post transplant hyperlipidemia	18 (31%)
Post transplant diabetes mellitus	11 (19%)
Post transplant hypertension	8 (13.8%)
History of rejection, N (%)	4 (6.9%)
DONORS (n=53)	
Age of kidney donor at the time of transplantation [years, mean±SD (range)]	33.17 ± 9.3 (19-62)
Gender of kidney donor, N (%)	
Men	33 (56.9%)
Type of donor of last transplant, N (%)	
Living related	51 (87.9%)
Living unrelated	7 (12.1%)
Cadaveric	0 (0%)

* HD: hemodialysis, **CAD: coronary artery disease, ***SLE: systemic lupus erythematous

All patients included in this study were of the same ethnic group (Caucasians). All of the recipients were followed up immediately post-surgery and up to 6 months; follow up data of 3 recipients during month four, 5 recipients during month five and 6 recipients during month six post-transplantation were not available. All patients were maintained on triple therapy with tacrolimus as a base of immunosuppressive protocol, in addition to prednisolone, and one of antimetabolites; azathioprine or mycophenolate mofetil. The most commonly used medications were calcium channel blockers, n=51 (87.9%), beta-blockers, n=31 (53.4%), proton pump inhibitors (PPIs) (omeprazole), n=29 (50%), H2 receptor antagonists (famotidine), n=21 (36.2%), statins, n=19 (32.8%), hydralazine, n=17 (29.3%), insulin, n=13 (22.4%), furosemide, n=8 (13.8%), doxazocin, n=8 (13.8%).

Among 51 recipients using CCBs, 4 (6.9%) received amlodipine, 39 (67.2%) nifedipine, and 19 (32.8%) diltiazem.

2. Changes in tacrolimus doses and blood concentrations over the first 6 months post-transplantation

The mean tacrolimus trough concentrations, tacrolimus weight-adjusted doses and tacrolimus dose-adjusted trough levels during the first 6 months post kidney transplantation are shown in Table 10.

As seen in the table, during the first 3 months post-transplant, mean tacrolimus trough concentrations were higher than the target level (7-10 ng/ml); progressively declining towards month 4, after which the average tacrolimus trough concentrations remained stable within the target level. Tacrolimus doses were progressively decreasing over six months post transplantation. Tacrolimus dose-adjusted trough levels were steadily increasing till the third month post transplantation after which they remained relatively stable.

Table 10: Mean tacrolimus concentrations, doses and dose-adjusted levels during the first six months in Jordanian kidney transplant recipients (n=58)

Parameter, Mean ± SD (Range)	**Treatment period post transplantation (months)**					
	1 (n=55)	**2 (n=58)**	**3 (n=56)**	**4 (n=55)**	**5 (n=48)**	**6 (n=51)**
Tacrolimus trough concentration (ng/ml)	15.27±4.72 (8.65-30)	13.18±3.27 (8.2-20.33)	10.56±2.33 (6.77-20.45)	9.95±2.37 (6.6-15.7)	9.94±2.39 (5.8-16)	9.21±2.44 (4.9-19)
*P**		0.001	<0.001	0.02	0.84	0.041
Tacrolimus weight-adjusted dose (mg/kg/day)	0.19±0.03 (0.11-0.27)	0.15±0.05 (0.05-0.29)	0.11±0.05 (0.04-0.23)	0.095±0.04 (0.03-0.18)	0.083±0.04 (0.03-0.18)	0.077±0.04 (0.03-0.17)
*P**		<0.001	<0.001	<0.001	<0.001	<0.001
Tacrolimus dose-adjusted trough level (ng/ml per mg/ kg /day)	83.91±33.02 (39.4-175.1)	102.86±51.78 (34.3-238.5)	118.3±70.25 (36.1-287.2)	126.32±64.4 (44.3-360)	145.8±78.5 (54-341)	146.1±82.01 (46.3-374.5)
*P**		0.001	0.006	0.299	0.021	0.511

*Paired t-test, compared to the previous month.

3. Genotyping and allelic frequencies

Among 53 kidneys transplant donors, 28 (52.8 %) were homozygote wild type (*GG*), 20 (37.7%) were heterozygotes mutant (*GT*), and the remaining 5 (9.4%) were homozygote mutant type (*TT*), as shown in Table 11. Figure 7 shows PCR product and restriction fragment length polymorphism (RFLP) analysis of *MDR1 G2677T/A* polymorphism.

Table 11: Frequencies of *G2677T/A* polymorphism in 53 Jordanian kidney transplant donors

Genotype	Number of subjects	%
2677GG	28	52.8 %
2677GT	20	37.7 %
2677TT	5	9.4 %
Allele frequency		
G allele	72 %	
T allele	28 %	

Using Hardy-Weinberg equilibrium (H-W), there was no significant difference between the current study finding and H-W expectation (*p*-value >0.05) by Chi-Square (Table 12).

Table 12: Comparing genotyping results in present study with H-W expectation.

	Genotype		
	GG	***GT***	***TT***
Present study	28	20	5
Hardy-Weinberg*	27	22	4
p**	0.26		

* H-W calculations are shown in Appendix VI, ** Chi-Square

Genotype and allelic frequencies of *ABCB-1* in this study match reported results for Caucasian populations (Table 13).

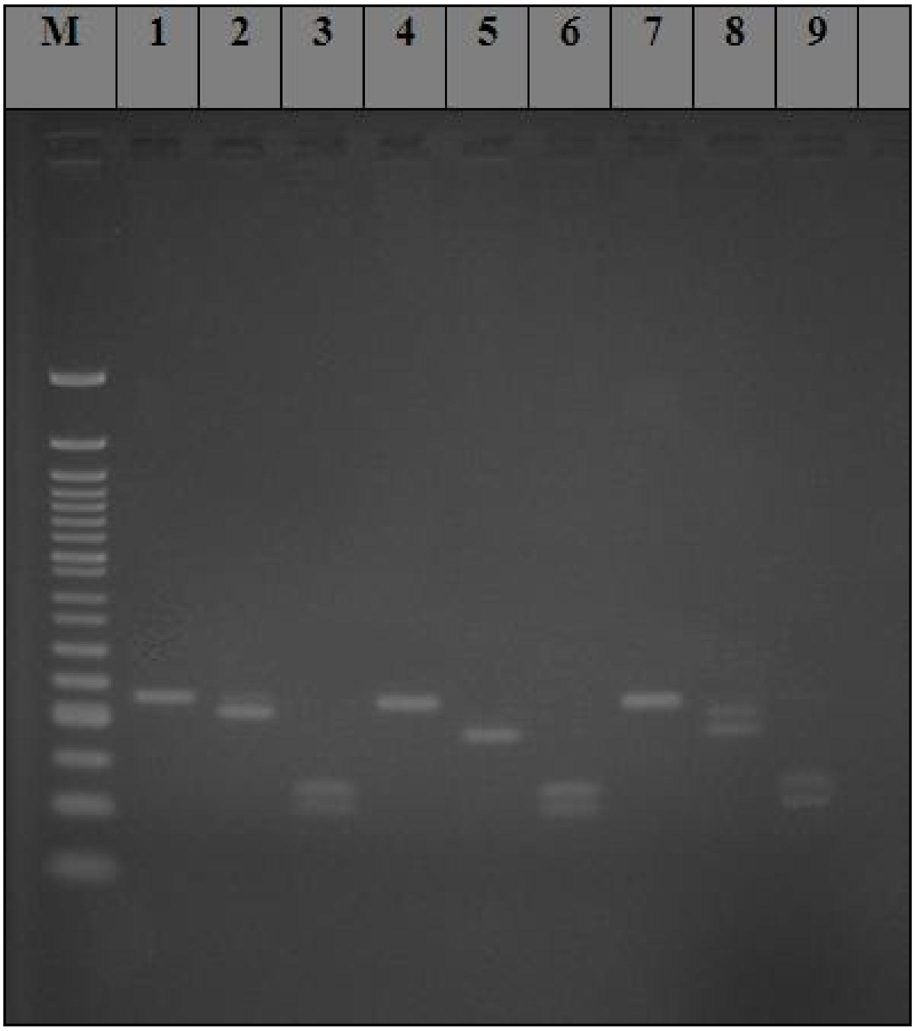


Figure 7: 2% Agarose gel electrophoresis (0.5X TBE), undigested PCR and RFLP product of *MDR1 G2677T/A* allele. Lane M: 50 bp DNA step ladder; Lane 1, 4, and 7: undigested PCR product (229 bp) for 3 samples; Lane 2: *Ban 1* digested RFLP product (209 bp); Lane 3: *Rsa 1* digested RFLP product (123 & 106 bp); Lanes 2 & 3: Homozygote mutant type *TT*; Lane 5: *Ban 1* digested RFLP product (186 bp); Lane 6: *Rsa 1* digested RFLP product (123 & 106 bp); Lanes 5 & 6: Wild type *GG*; Lane 8: *Ban 1* digested RFLP product (209, 186 bp); Lane 9: *Rsa 1* digested RFLP product (123 & 106 bp); Lanes 8 & 9: Heterozygote mutant type *GT*.

Table 13: Frequencies of *G2677T/A* polymorphism in *ABCB-1* gene in selected populations

Population (Sample Size)	*G* alleles	*T* alleles	*A* alleles	*GG*	*TT*	*GT*	*AA*	*AT*	*GA*	Reference
Jordanian (53)	(72) 72%	(28) 28%	0	(28) 52.8%	(5) 9.4%	(20) 37.7%	0	0	0	Present study
Jordanian (43)	(59) 68.6%	(13) 27%	0	(21) 48.8%	(5) 11.7	(17) 39.5%	0	0	0	Ulemat, 2010
Jordanian (96)	(131) 76.2%	(41) 23.8%	0	(49) 51%	(10) 10.4%	(37) 38.6%	0	0	0	Composite
Caucasian (67)	(75) 56%	(59) 44%	0	(25) 36.9%	(17) 26.2%	(25) 36.9%	0	0	0	Innocenti, *et. al*., 2009
Caucasian (32)	(59) 92%	(5) 8%	0	(27) 84%	0	(5) 16%	0	0	0	Provenzani, *et. al*., 2009
Caucasian (280)	(350) 63%	(197) 35%	(13) 2%	(110) 39.5%	(34) 12%	(123) 44%	0	(6) 2%	(7) 2.5%	Gonzalez, *et. al*., 2008
Caucasian white (24)	(35) 73%	(13) 27%	0	(12) 50%	(1) 4%	(11) 46%	0	0	0	Mourad, *et. al*., 2005
Caucasian (50)	(65) 65%	(32) 32%	(3) 3%	(23) 46%	(8) 16%	(16) 32%	0	0	(3) 6%	Haufroid, *et. al*., 2004
Caucasian (453)	(584) 64.4%	(306) 33.8%	(16) 1.8%	(197) 43.5%	(60) 13.2%	(180) 39.7%	0	(6) 1.3%	(10) 2.2%	Composite
Japanese (181)	(147) 40%	(168) 46%	(47) 14%	(30) 16.6%	(32) 17.7%	(73) 40.3%	(1) 0.6%	(31) 17.1%	(14) 7.7%	Goto, *et. al*., 2004
Japanese (100)	(43) 43%	(39) 39%	(18) 18%	(14) 14%	(16) 16%	(35) 35%	(1) 1%	(11) 11%	(23) 23%	Tanabe, *et. al*., 2001
Japanese (281)	(190) 41.1%	(207) 44.8%	(65) 14%	(44) 15.7%	(48) 17%	(108) 38.4%	(2) 0.7%	(42) 14.9%	(37) 13.2%	Composite

4. Donor's *MDR1 G2677T/A* genotype and tacrolimus dose requirements

Dose-adjusted trough level (ng/ml per mg/kg/body weight), daily dose (mg/kg body weight), and trough concentration (ng/ml) of tacrolimus were compared among recipients of donors with different allelic status of *MDR1 G2677T/A*: recipients of donors carrying the *GG* genotype (homozygous wild type) and of those carrying at least one *T* allele (*TT* or *GT*) (homo- and heterozygous mutant type, respectively). Tacrolimus trough level, dose and dose-adjusted level did not differ significantly between different *MDR1 G2677T/A* genotype groups during the first 6 months post transplant (Table 14, Figures 8-13).

Table 14: Relation between donor's *MDR1 G2677T/A* genotype (n=53) and tacrolimus trough level, dose and dose-adjusted level during the first 6 months post transplantation

Month	**Tacrolimus pharmacokinetic parameter, mean ± SD**								
	Trough level (ng/ml)			Weight-adjusted dose (mg/kg/day)			Dose-adjusted trough level (ng/ml per mg/kg/day)		
	Donor's genotype			**Donor's genotype**			**Donor's genotype**		
	GG*	***TT/GT*****	***P*****	***GG****	***TT/GT*****	***P*****	***GG****	***TT/GT*****	***P*****
1	14.6±3.6 (n=26)	15.7±5.7 (n=24)	0.40	0.18±0.03	0.19±0.02	0.67	81.9±28.6	86.8±38.9	0.62
2	13.0±4.0 (n=28)	13.5±2.6 (n=25)	0.62	0.15±0.05	0.15±0.05	0.91	101±52.3	108.5±55.8	0.62
3	10.3±1.8 (n=27)	10.9±3.03 (n=24)	0.42	0.12±0.05	0.10±0.05	0.24	106.5±63.3	134.9±81.3	0.17
4	10.1±2.4 (n=27)	9.7±2.4 (n=23)	0.60	0.1±0.04	0.09±0.05	0.51	116.5±52.8	138.8±81.3	0.25
5	9.95±2.2 (n=24)	10.02±2.6 (n=20)	0.93	0.09±0.04	0.08±0.04	0.51	132.6±56.9	169.7±100	0.13
6	9.05±2.04 (n=26)	9.4±2.98 (n=20)	0.66	0.08±0.04	0.08±0.04	0.90	136.1±64.6	164.9±106.1	0.26

* Homozygote wild type, **mutant (homo & heterozygote carrying at least one *T*), *** t- test

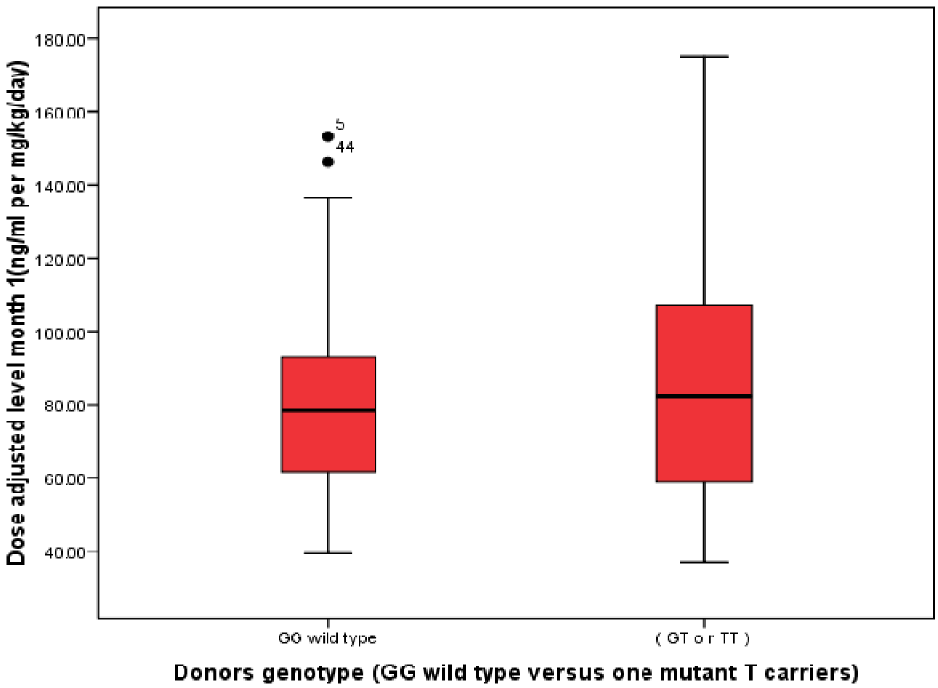


Figure 8: Box plot of tacrolimus dose-adjusted trough level in kidney transplant recipients versus donors' *MDR1 G2677T/A* genotype, month 1.

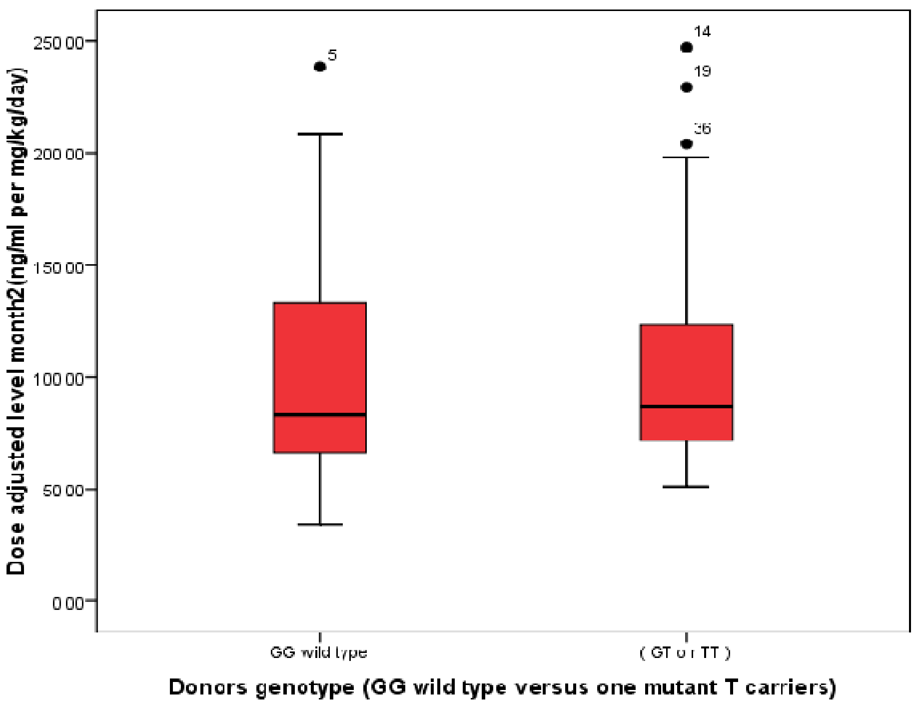


Figure 9: Box plot of tacrolimus dose-adjusted trough level in kidney transplant recipients versus donors' *MDR1 G2677T/A* genotype, month 2.

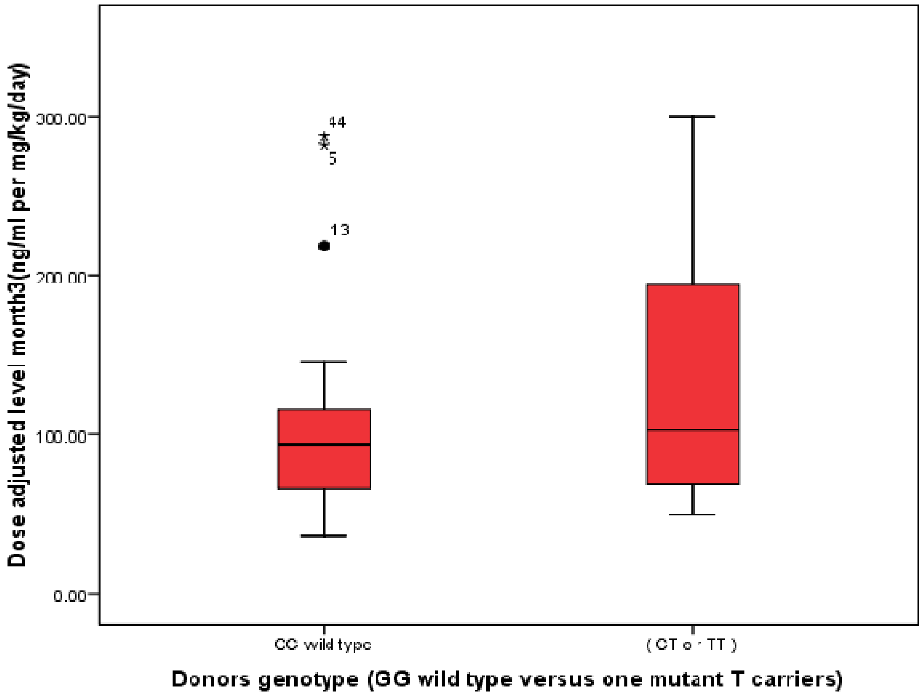


Figure 10: Box plot of tacrolimus dose-adjusted trough level in kidney transplant recipients versus donors' *MDR1 G2677T/A* genotype, month 3.

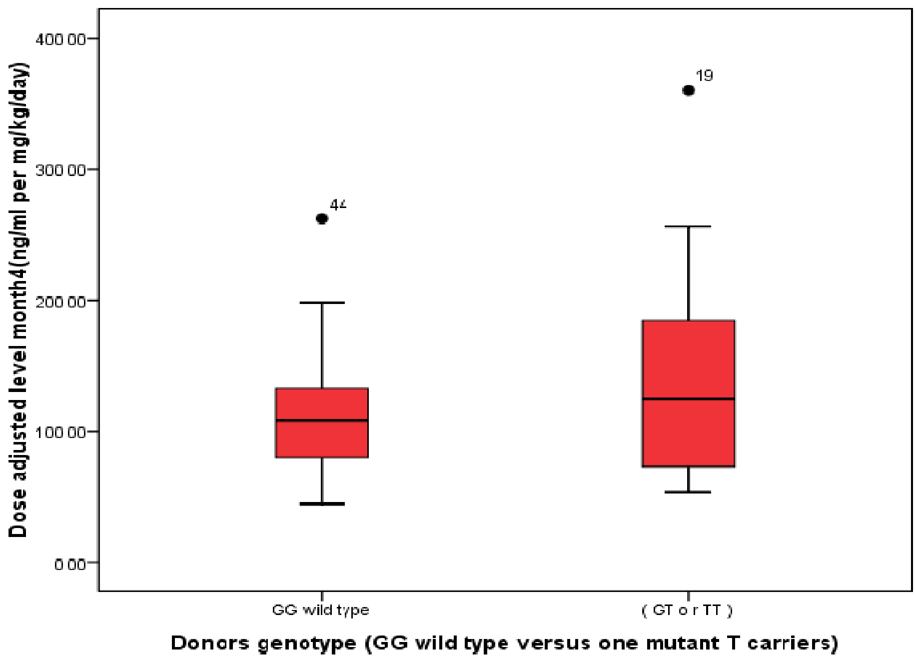


Figure 11: Box plot of tacrolimus dose-adjusted trough level in kidney transplant recipients versus donors' *MDR1 G2677T/A* genotype, month 4.

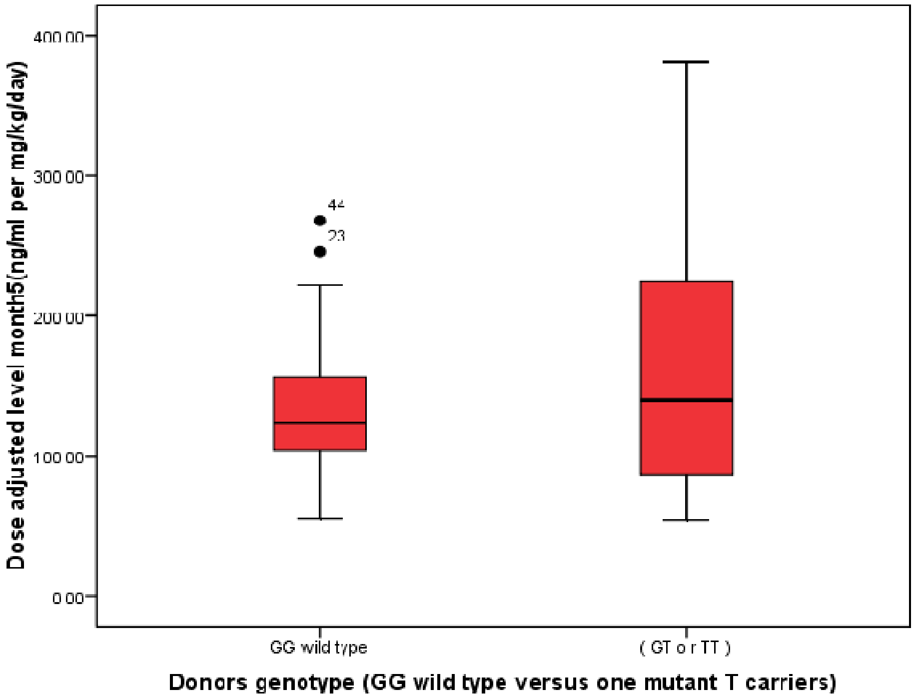


Figure 12: Box plot of tacrolimus dose-adjusted trough level in kidney transplant recipients versus donors' *MDR1 G2677T/A* genotype, month 5.

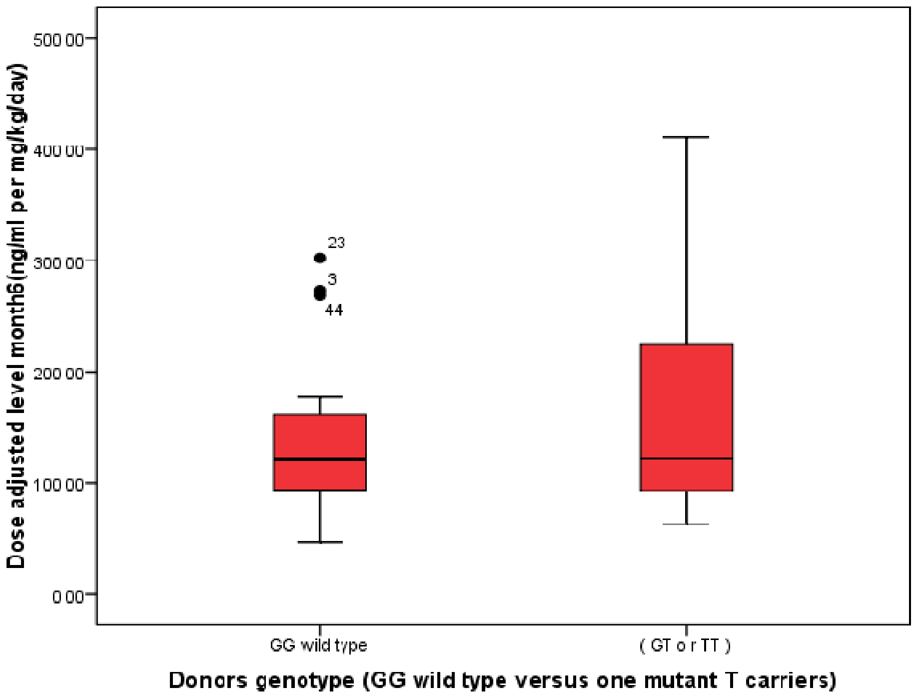


Figure 13: Box plot of tacrolimus dose-adjusted trough level in kidney transplant recipients versus donors' *MDR1 G2677T/A* genotype, month 6.

5. Gender differences in relationship between donor's *MDR1 G2677T/A* genotype and tacrolimus dose requirements

Using independent-samples t-test, tacrolimus trough concentration, weight-adjusted dose and dose–adjusted trough level were compared among recipients of donors with different allelic status of *MDR1 G2677T/A* during the first 6 months post transplantation by recipients' gender (Table 16). No significant differences in tacrolimus trough level, weight-adjusted dose and dose-adjusted level were observed in Jordanian male and female kidney transplant recipients when tested by donors *MDR1 G2677T/A* genotype groups.

Table 15: Relation of donor *MDR1 G2677T/A* genotype (n=53) and tacrolimus trough level, dose and dose-adjusted level in Jordanian kidney transplant during the first 6 months post transplantation according to recipients' gender.

Month	Recipients gender	Tacrolimus trough level (ng/ml), mean ± SD			Tacrolimus weight-adjusted dose (mg/kg/day), mean ± SD			Dose adjusted trough level (ng/ml per mg/kg/day), mean ± SD		
		Donors genotype			Donors genotype			Donors genotype		
		*GG**	*TT/GT***	*P****	*GG**	*TT/GT***	*P****	*GG**	*TT/GT***	*P****
1	Men	15.8±3.93 (n=13)	15.91±6.29 (n=17)	0.96	0.17±0.03	0.19±0.02	0.14	94.1±32.2	87.0±38.6	0.59
	Women	13.38±2.86 (n=13)	15.28±4.13 (n=7)	0.24	0.196±0.02	0.190±0.03	0.71	69.7±18.7	86.1±42.8	0.25
2	Men	13.5±4.66 (n=15)	13.9±2.8 (n=18)	0.80	0.13±0.04	0.14±0.05	0.66	115.2±57.6	116.3±57.1	0.96
	Women	12.39±3.13 (n=13)	12.46±1.49 (n=7)	0.96	0.17±0.05	0.16±0.05	0.93	84.67±41.69	88.63±50.71	0.85
3	Men	10.9±1.4 (n=14)	10.5±2.3 (n=18)	0.61	0.10±0.04	0.097±0.04	0.73	131.3±75.8	141.3±81.1	0.72
	Women	9.66±1.9 (n=13)	11.83±4.8 (n=6)	0.17	0.14±0.05	0.12±0.04	0.49	79.68±31.2	115.7±86.5	0.19
4	Men	10.2±9.3 (n=15)	9.3±1.6 (n=17)	0.28	0.09±0.04	0.08±0.05	0.75	131.2±55.8	148.6±84.6	0.50
	Women	9.91±1.72 (n=12)	10.91±3.69 (n=6)	0.44	0.11±0.04	0.11±0.04	0.98	98.3±41.4	111.2±70.2	0.63
5	Men	10.1±2.8 (n=14)	9.7±2.7 (n=15)	0.68	0.066±0.03	0.071±0.04	0.69	156.3±75.5	167.2±102.9	0.75
	Women	9.77±1.12 (n=10)	11.1±2.55 (n=5)	0.19	0.10±0.04	0.10±0.05	0.98	109.3±47.9	135.9±83.4	0.54
6	Men	9.05±2.04 (n=26)	9.4±2.98 (n=20)	0.66	0.08±0.04	0.08±0.04	0.90	136.1±64.6	164.9±106.1	0.26
	Women	9.25±1.79 (n=12)	10.48±1.98 (n=5)	0.23	0.09±0.04	0.09±0.05	0.96	112.6±40.2	158.1±127.9	0.27

* Homozygote wild type, **mutant (homo & heterozygote carrying at least one *T*), *** independent sample t- test

6. Donor's *MDR1G2677T/A* genotype and renal function in kidney recipients

Serum creatinine concentration and creatinine clearance were compared among kidney recipients with different donor's allelic status of *MDR1 G2677T/A*. Independent-samples t-test was used for this analysis and data are shown in Table 15. No significant differences in renal function were found between different *MDR1 G2677T/A* genotype groups during the first 6 months post transplantation**.**

Table 16: Relation of donors' *MDR1 G2677T/A* genotype to renal function in Jordanian kidney transplant recipients during the first 6 months post transplantation

Month	**Parameter,** mean ± SD	**Donor's *MDR1* genotype**		***P****
		***GG* Wild type**	**At least one *T(TT or GT*)**	
1	Serum creatinine (mg/dl)	1.3 ± 0.53 (n=28)	1.43 ± 1.05 (n=25)	0.61
	Creatinine clearance (ml/min)	65.11 ± 18.53	68.08 ± 21.58	0.59
2	Serum creatinine (mg/dl)	1.21 ± 0.29 (n=28)	1.2 ± 0.23 (n=25)	0.85
	Creatinine clearance (ml/min)	68.32 ± 16.59	70.36 ± 13.46	0.63
3	Serum creatinine (mg/dl)	1.26 ± 0.42 (n=28)	1.16 ± 0.21 (n=25)	0.30
	Creatinine clearance (ml/min)	67.86 ± 20.28	71.17 ± 10.93	0.48
4	Serum creatinine (mg/dl)	1.24 ± 0.37 (n=27)	1.26 ± 0.35 (n=23)	0.84
	Creatinine clearance (ml/min)	69.3 ± 23.04	69.4 ± 20.57	0.99
5	Serum creatinine (mg/dl)	1.23 ± 0.31 (n=26)	1.25 ± 0.24 (n=22)	0.85
	Creatinine clearance (ml/min)	66.6 ± 17.2	68.95 ± 18.63	0.65
6	Serum creatinine (mg/dl)	1.22 ± 0.29 (n=26)	1.27 ± 0.25 (n=21)	0.55
	Creatinine clearance (ml/min)	67.27 ± 17.2	67.0 ± 12.23	0.95

* independent-samples t- test

7. Impact of CCBs (diltiazem) on tacrolimus blood level, dose and dose-adjusted level in kidney transplant recipients

To compare tacrolimus blood level, dose and dose-adjusted level in kidney transplant recipients who were prescribed diltiazem and those without diltiazem; we used independent-samples t-test (Table 17). Diltiazem had no significant effect on tacrolimus trough levels, dose and dose-adjusted trough level.

Table 17: Relation of diltiazem use and tacrolimus trough level, dose and dose-adjusted level in Jordanian kidney transplant recipients during the first 6 months post transplantation

Month	**Parameter,** mean ± SD	**Diltiazem prescribed**		***P****
		Yes (n=19)	No (n=39)	
1	Tacrolimus trough level (ng/ml)	15.66 ± 3.92	14.64 ± 5.8	0.44
	Tacrolimus dose (mg/kg/day)	0.19 ± 0.02	0.18 ± 0.03	0.48
	Dose-adjusted trough level (ng/ml per mg/kg/day)	83.7 ± 25.4	84.2 ± 43.4	0.96
2	Tacrolimus trough level (ng/ml)	13.31±3.53	12.98±2.87	0.72
	Tacrolimus dose (mg/kg/day)	0.149±0.05	0.146±0.05	0.77
	Dose-adjusted trough level (ng/ml per mg/kg/day)	101.6±48.77	104.9±57.49	0.81
3	Tacrolimus trough level (ng/ml)	10.4±1.85	10.8±2.95	0.53
	Tacrolimus dose (mg/kg/day)	0.11±0.05	0.11±0.05	0.86
	Dose-adjusted trough level (ng/ml per mg/ kg/day)	112.96±63.1	126.5±80.99	0.48
4	Tacrolimus trough level (ng/ml)	10±2.55	9.87±2.11	0.84
	Tacrolimus dose (mg/kg/day)	0.094±0.04	0.096±0.04	0.82
	Dose-adjusted trough level (ng/ml per mg/ kg/day)	122.2±49.1	133.1±84.5	0.55
5	Tacrolimus trough level (ng/ml)	9.86±2.37	10.1±2.48	0.76
	Tacrolimus dose (mg/kg/day)	0.084±0.04	0.082±0.04	0.79
	Dose-adjusted trough level (ng/ml per mg/ kg/day)	138.7±74.2	158.2±83.4	0.40
6	Tacrolimus trough level (ng/ml)	9.52±2.6	8.69±2.1	0.24
	Tacrolimus dose (mg/kg/day)	0.08±0.03	0.08±0.04	0.77
	Dose-adjusted trough level (ng/ml per mg/ kg/day)	148.68±81.5	141.79±84.9	0.77

* independent-samples t-test

8. Further analysis

Independent-samples t-test and Chi square tests were used to compare demographic and clinical variables of donors and recipients according to donor's *MDR1 G2677T/A* allelic status. Table 18 show that there were no significant differences in recipient's gender, age, weight, body mass index, donor gender, gender match between donor and recipient, corticosteroid dose, CCBs use, albumin or hematocrit levels, suggesting that these factors did not have impact on difference in tacrolimus blood level between the two genotype-based groups (donor *GG ABCB1* genotype versus at least one *T* (*GT* or *TT* *ABCB1* genotype). Kolmogorov-Smirnov Z test was used to test normality of each variable group used in our study. As shown in Table 19, all data were normally distributed.

Table 18: Relation of donor's *MDR1 G2677T/A* genotype to demographic and clinical variables in donors and recipients.

Parameter	Donors *MDR1 G2677T/A* genotype (n=53)		*P*
	***GG –Wild* (n=28)**	***TT/GT mutant. At least one T* (n=25)**	
Age (years)	36.4 ± 11.4	35.3 ± 9.1	0.71*
Weight (kg)	68.2 ± 16.4	70.2 ± 14.1	0.64*
Body Mass Index (kg/m^2)	24.6 ± 4.8	24.3± 4.8	0.85*
Albumin, month 1	12.0 ± 1.7	12.4 ± 1.9	0.57*
Albumin, month 2	14.4 ± 6.9	13.2 ± 1.7	0.47*
Albumin, month 3	12.8 ± 1.6	13.2 ± 1.4	0.42*
Albumin, month 4	12.9 ± 1.3	13.3 ± 1.7	0.42*
Albumin, month 5	13.3 ± 1.7	13.7 ± 1.4	0.55*
Albumin, month 6	13.3 ± 1.7	13.7 ± 1.3	0.42*
Hematocrit, month 1	37.0 ± 4.9	36.1 ± 5.9	0.56*
Hematocrit, month 2	38.3 ± 4.4	38.2 ± 4.8	0.98*
Hematocrit, month 3	38.4 ± 5.0	39.2 ± 4.3	0.52*
Hematocrit, month 4	39.6 ± 3.8	39.2 ± 5.0	0.75*
Hematocrit, month 5	40.7 ± 4.8	40.6 ± 5.4	0.94*
Hematocrit, month 6	41.7 ± 4.3	41.8 ± 5.1	0.96*
Prednisolone dose	15.98 ± 5.1	14.88 ± 5.6	0.46*
Recipient gender observed (expected)	M: 15 (17.4) F: 13 (10.6)	M: 18 (15.6) F: 7 (9.4)	0.17**
Donor gender observed (expected)	M: 14 (15.8) F: 14 (12.2)	M: 16 (14.2) F: 9 (10.8)	0.31**
Gender match between donor and recipient observed (expected)	M to M: 7 (9.5) M to F: 7 (6.3) F to M: 8 (7.9) F to F: 6 (4.2)	M to M: 11 (8.5) M to F: 5 (5.7) F to M: 7 (7.1) F to F: 2 (3.8)	0.37**
Diltiazem use observed (expected)	Yes: 10 (11.1) No:18(16.9)	Yes: 11 (9.9) No: 14 (15.1)	0.54**

Data represented as mean ± SD, *independent-samples t- test. ** Chi square test.

Table 19: Normality of distributions in demographic and clinical variables in donors and recipients groups

Variable	Kolmogorov-Smirnov Z significance
Tacrolimus trough concentration (ng/ml) month 1	0.611
Tacrolimus weight-adjusted dose (mg/kg/day) month 1	0.421
Dose-adjusted tacrolimus concentration (ng/ml per mg/ kg/day) month 1	0.166
Tacrolimus trough concentration (ng/ml) month 2	0.097
Tacrolimus weight-adjusted dose (mg/kg/day) month 2	0.975
Dose-adjusted tacrolimus concentration (ng/ml per mg/ kg/day) month 2	0.088
Tacrolimus trough concentration (ng/ml) month 3	0.348
Tacrolimus weight-adjusted dose (mg/kg/day) month 3	0.638
Dose-adjusted tacrolimus concentration (ng/ml per mg/ kg/day) month 3	0.005
Tacrolimus trough concentration (ng/ml) month 4	0.354
Tacrolimus weight-adjusted dose (mg/kg/day) month 4	0.532
Dose-adjusted tacrolimus concentration (ng/ml per mg/ kg/day) month 4	0.102
Tacrolimus trough concentration (ng/ml) month 5	0.390
Tacrolimus weight-adjusted dose (mg/kg/day) month 5	0.163
Dose-adjusted tacrolimus concentration (ng/ml per mg/ kg/day) month 5	0.058
Tacrolimus trough concentration (ng/ml) month 6	0.095
Tacrolimus weight-adjusted dose (mg/kg/day) month 6	0.138
Dose-adjusted tacrolimus concentration (ng/ml per mg/ kg/day) month 6	0.050
Recipient age (years)	0.427
Weight (kg)	0.871
Body mass index (kg/cm.cm)	0.943

V. DISCUSSION

The clinical use of tacrolimus is complicated by its narrow therapeutic range and highly variable pharmacokinetics among various individuals. Some patients do not reach target concentrations using recommended initial doses of tacrolimus. They, therefore, have an increased risk of under immunosuppression and acute rejection during the early period post organ transplantation. The association of the *ABCB1* gene SNP with tacrolimus dose requirements has been recognized as a genetic basis for the observed inter individual differences in pharmacokinetics (Macphee, *et al*., 2002; Anglicheau, *et al*., 2003; Hesselink*, et al.,* 2003; Tada, *et al.,* 2005).

In our study, *MDR-1* alleles frequency (*G* allele: 72%, *T* allele: 28%) is consistent with data previously studied for Jordanian population (Ulemat, 2010), and with data previously published for Caucasian population (Haufroid, *et al*., 2004; Mourad, *et al*., 2005; Gonzalez, *et al*., 2008; Innocenti, *et al*., 2009; Provenzani, *et al*., 2009). Genetic polymorphisms of *MDR1* may be important for tacrolimus pharmacokinetics since P-gp, the *MDR1* product, is an important transport protein known to be involved in tacrolimus absorption in the gut, distribution across the body, metabolism and excretion (Li, D.*, et al.*, 2006; Taubert, *et al.,* 2006).

The proximal tubule in kidney plays an important role in the tubular secretion of tacrolimus. Tubular secretion can be considered a three-step process consisting of uptake across the basolateral membrane, intracellular accumulation, and efflux across the apical membrane. The uptake and efflux steps are mediated by a range of transport proteins located at the basolateral and apical membranes of proximal tubule cells, which act as an effective secretory mechanism (Colin, *et al*., 2008; Verhulst, *et al*., 2008).

Kim *et al.* (1998) demonstrated, using a retrovirus expression system, that cells expressing the (*T*; Serine; mutant) P-gp variant had roughly a two-fold increased

transport activity for digoxin relative to the corresponding cells expressing the reference protein (*G*; Alanine; wild). Later, Kim *et al.* (2001) found that the *2677TT* (mutant) genotype was associated with significantly lower plasma concentrations of the P-gp substrate fexofenadine, which is explained by a higher intestinal P-gp expression.

On the other hand, it was reported that the *G* allele of *G2677A/T* polymorphism was associated with a higher expression level of P-glycoprotein in the placenta compared with other genotypes (Tanabe, *et al.,* 2001).

An important relation was noted for the exon 21 *G2677T/A* SNP in 81 renal transplant recipients, most of whom were Caucasian: tacrolimus dose requirement was 40% higher, and the concentration/dose ratio 36% lower in homozygous mutant than wild-type carriers in renal transplant recipients at 1 month post transplantation (Anglicheau, *et al*., 2003). Another study found that recipients carrying the (*T* or *A*) mutation had tacrolimus concentrations 44.7% higher than that in the wild-type individuals (Mendes, *et al*., 2009).

In 86 Chinese renal transplant recipients, the *MDR1 G2677T/A* and *C3435T* gene polymorphisms were correlated with the whole blood concentration of tacrolimus, and in order to obtain similar blood concentration, patients with *2677GG* and *3435CC* genotype carriers should take the drug at a higher dose than those with *3435CT, 3435 TT*, or *2677 TT* at three, six, and twelve months post transplantation (Wang, *et al.* 2005). Among *3435T* allele carriers, a weak association was noted between recipients' *ABCB1* polymorphism in exon 26 and tacrolimus dose requirements at 3 months after renal transplantation (Macphee, *et al*., 2002). The homozygous carriers of the *3435T* allele showed on average more than a twofold lower intestinal P-gp expression level compared to the *CC* genotype (Haufroid, *et al*., 2004).

Wavamunno & Chapman (2008) emphasized a need for prospective studies to explore the impact of genetic polymorphism of transport proteins in kidney donors that play an important role in recipient's drug pharmacokinetics. Such studies are anticipated to improve utility of donor and recipient genotype testing in managing immunosuppression therapy.

In a previous study in 57 Jordanian renal transplant recipients during stable post transplant period, trough tacrolimus concentrations in recipients of donors carrying at least one *T* mutant alleles (*2677TT* or *2677GT*) showed 27% higher trough concentration than recipients of donors carrying homozygote wild *2677GG* genotype. There was no significant difference in tacrolimus dose and tacrolimus dose-adjusted trough concentration between the two groups (Ulemat, 2010).

The present study found a no significant relationship between *G2677T/A* donor's genotype and tacrolimus trough concentration in kidney transplant recipients during the early post-transplant period. Tacrolimus dose-adjusted trough concentration was not significantly different between patients who obtained kidney from donors with at least one mutant *T* allele and those who obtained kidney from donors with wild type allele.

Study of demographic and clinical variables in donors and recipients showed that there were no significant differences between the recipient groups based on donor *MDR1* genotype regarding recipient's age, weight, BMI, albumin concentration, hematocrit concentration, corticosteroid dose, recipient's and donor's gender, gender match between donor and recipient, and use of diltiazem, suggesting that these factors did not interfere with the impact of *MDR1* polymorphism on tacrolimus blood level.

Potential justifications may explain the controversy of results between the published findings and findings of our study, including:

- The published studies performed in different types of solid organ transplantation, at different periods after transplantation, with different methods used to measure drug concentrations and some studies might have been statistically underpowered.
- Differences in other genes involved in the pharmacokinetics of tacrolimus.
- Conflicting results concerning alterations in P-gp expression and function among *GG* carriers or *GT/TT* carriers may suggest that sometimes the observed effect is not directly related to *G2677T/A* genotype, rather it is a reflection to other related SNPs at different sites (Sakeada, 2005; Yan-Hong, 2005; Zhou, 2008).
- Multiple studies have demonstrated a linkage disequilibrium between the non-synonymous polymorphism in exon 21 and other *SNPs* in *MDR1* including the exons *26 (C3435T)* and *12 (C1236T)*, suggesting that the functional effects may be haplotype-dependent. Since the number of relevant haplotypes in *MDR1* has been shown to significantly differ in various populations based on racial ancestry, determination of haplotype may prove to be important when assessing the effects of *MDR-1 SNPs* to in vivo functional consequences (Marzolini, *et al.*, 2005). Unfortunately, the effects of other SNPs were not investigated in our study, and haplotype analysis of *MDR1* may be a superior method to analyze the effect of polymorphisms in this gene on the tacrolimus pharmacokinetics.
- Differences in *CYP3A* genotype between donors and recipients could affect tacrolimus metabolism.
- Renal transplant patients receive many medications that may alter P-gp expression and function thus, acting as inhibitors or inducers for tacrolimus.

The mean tacrolimus blood levels during month 1, 2 and 3 post transplantation were (15.27±4.72, 13.18±3.27, 10.56±2.33 ng/mL, respectively) being above the recommended range of (7-10 ng/ml), while during month 4, 5 and 6 levels were (9.95±2.37, 9.94 ±2.39 and 9.21±2.44 ng/mL, respectively) within the recommended range. Our results show that the mean doses required to achieve target levels of tacrolimus during the early 6 months were (0.19, 0.15, 0.11, 0.095, 0.083 and 0.077 mg/kg/day, respectively) steadily decreasing during the first six months post transplantation. During the first 3 months post transplant doses were within recommended range (0.15–0.3 mg/kg/day) (Staatz and Tett, 2004) and very similar to doses used in European populations, [0.12 and 0.168 mg/kg/day (Margretier, 2002 and Thervet, *et al*., 2008), respectively] or American population (0.1 mg/kg/day) (Macphee, *et al.*, 2002). However, doses during the period of 4-6 months in our study were lower than the recommended dose.

The interactions between tacrolimus and diltiazem are believed to be complex due to diltiazem being substrate, inhibitor or even inducer of *MDR1* protein, in addition to competitive inhibition of *CYP3A* (Zhou, 2008). In our study, there was a lack of statistically significant effect of diltiazem on tacrolimus dose requirements and dose-adjusted level, even after dividing patients by donor's *MDR1* genotype.

Changes in activity and expression of drug metabolizing enzymes, and/or modulation of active drug transport systems (e.g. P-gp) by sexual steroids may cause partly gender differences in drug action (Bies, *et al.*, 2003; Fröhlich*, et. al*., 2004). Hosohata K (2009) found that intestinal *CYP3A4 mRNA* expression levels showed significantly higher values in women, but not in men carrying the *2677TT-3435TT* haplotype than those with *2677GG-3435CC* and *2677GT-3435CT* haplotypes. There was no significant difference in tacrolimus trough level, dose and dose-adjusted level between different

Jordanian male or female renal transplant recipients stratified by donor *MDR1 G2677T/A* genotype.

VI. CONCLUSIONS

- Among the study population, the allelic frequency of *2677GG* allele is 52.8%, *2677GT* allele is 37.7%, and *2677TT* allele is 9.4%, consistent with other Caucasians.
- There is no significant difference in tacrolimus trough levels in kidney recipients of donors carrying *2677GT* or *2677TT* alleles compared to those recipients who obtained kidneys from donors carrying *2677GG* alleles during the early period post transplantation.
- The impact of the kidney donors *MDR1* 2677G>T/A polymorphism on tacrolimus pharmacokinetics is insufficient to have a significant effect on clinical outcome of Jordanian renal transplant patients during early post transplant period.
- Doses of tacrolimus that are necessary to achieve therapeutic levels among Jordanian kidney transplant recipients during the months 1, 2 and 3 post transplant are similar to the recommended doses and to those used in Western and American population, while during the months 4, 5 and 6 are lower than the recommended doses.
- To establish the predictive role of the donor's *MDR1* gene polymorphism in tacrolimus pharmacokinetics, there is a need for a large multicenter prospective trial assessing the impact of different individual SNPs and haplotypes in a standard fashion in renal transplant patient population with corresponding donors that is uniformly treated and systematically evaluated at different periods post transplantation.

VII. STUDY LIMITATIONS

1. The number of study patients was small because of the high per patient cost of DNA technologies and the long follow up period of 6 months needed per patient.
2. Because of the small sample size, we could not detect rare mutations and their impact on tacrolimus pharmacokinetic parameters, but our sample size matches other previously published studies.
3. Impact of other SNPs of the same gene and multi-genetic influences on tacrolimus pharmacokinetics and pharmacodynamics were not studied in the same population.

VIII. FUTURE WORKS

1. Increase the sample size of the study by involving more Jordanian kidney transplant recipients and their corresponding donors.
2. Extend the scope of the study to include pharmacodynamics of tacrolimus.
3. Assess the role of non-genetic factors on pharmacokinetics and pharmacodynamics of tacrolimus.
4. Investigate the combined effect of different SNPs in *MDR1* and *CYP3A* on the pharmacokinetics of tacrolimus, by using haplotype analysis.

REFERENCES

Alakulppi, N. Kyllönen, L. Jäntti, V. Matinlauri, I. Partanen, J. Salmela, K. and Laine, J. (2004), Cytokine gene polymorphisms and risks of acute rejection and delayed graft function after kidney transplantation. **Transplantation,** 78 (10), 1422-8

Anglicheau, D. Verstuyft, C. Laurent-Puig, P. Becquemont, L. Schlageter, M. Cassinat, B. Beaune, P. Legendre, C and Thevert, E. (2003), Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. **J Am Soc Nephrol**, 14, 1889–1896.

Balram, C. Sharma, A. Sivathasan, C. and Lee, E. J. (2003), Frequency of C3435T single nucleotide *MDR1* genetic polymorphism in an Asian population: phenotypic-genotypic correlates. **Br J Clin Pharmacol**, 56, 78–83.

Bekersky, I. Dressler, D. Alak, A. Boswell, G.W. and Mekki, QA. (2001), Comparative tacrolimus pharmacokinetics: normal versus mildly hepatically impaired subjects. **J Clin Pharmacol**, 41:628-35.

Berloco, P. Rossi, M. Pretagostini, R. Cortesini, N. and Cortesini, R. (2001), Tacrolimus as cornerstone immunosuppressant in kidney transplantation, **Transplant Proc**, 33, 994-996.

Bies, R. R. Bigos, K. L. and Pollock, B. G. (2003), Gender differences in the pharmacokinetics and pharmacodynamics of antidepressants. **J Gend Specif Med**, 6 (3), 12-20.

Borrows, R. Loucaidou, M. Van Tromp, J. Cairns, T. Griffith, M. Hakim, N. McLean, A. Palmer, A. Papalois, V. and Taube, D. (2004), Steroid sparing with tacrolimus and mycophenolate mofetil in renal transplantation. **Am J Transplant,** 4, 1845-1851.

Brinkmann, U. and Eichelbaum, M. (2001), Polymorphisms in the ABC drug transporter gene *MDR1*. **Pharmacogenomics J,** 1(1), 59-64.

Bulatova, N. Yousef, A-M. Al-Khayyat, G. and Qosa, H. (2011), Adverse effects of tacrolimus in renal transplant patients from living donors. **Curr Dr Safety**, 6 (1), 3-11.

Cavaco, I. Gil, J. P. Gil-Berglund, E. and Ribeiro, V. (2003), CYP3A4 and MDR1 alleles in a Portuguese population. **Clin. Chem. Lab. Med**. 41, 1345-1350.

Cheung, C. Wong, K. Wong, H. Liu, Y. Chan, Y. Wong, S. Chak, W. Choi, K. Chau, K. and Li, C. (2007), Influence of different allelic variants of the cytochrome 3A and adenosine triphosphate-binding cassette B1 gene on the tacrolimus pharmacokinetic profile of Chinese renal transplant recipients. **Pharmacogenomics**; 7:563-574.

Chinn, L. W. and Kroetz, D. L. (2007), *ABCB1* pharmacogenetics: progress, pitfalls, and promise. **Clin Pharmacol Ther**, 81(2), 265-9.

Chou, F. Tzeng, S. and Huang, J. (2001), Genetic polymorphism of cytochrome P450 *3A5* in Chinese, **Drug Metabolism and Disposition,** 29:1205–1209

Ciancio, G. Burke, G. Gaynor, J. Mattizzi, A. Roth, D. Kupin, W. Nicolas, M. Ruiz, P. Rosen, A. and Miller, J. (2004**)**, a randomized long-term trial of tacrolimus/sirolimus versus tacrolimus/mycophenolate mofetil versus cyclosporine (NEORAL)/ sirolimus in renal transplantation. **Transplant**, 77, 252-258.

Colin, D.A. Brown, R. S. Amy, S. W. Haslam, I. S. De Broe, M. E. D'Haese, P. C. and Verhulst, A. (2008), Characterization of human tubular cell monolayers as a model of proximal tubular xenobiotic handling. **Toxicol Appl Pharmacol**, 233, 428–438.

Comstock, T. (2002), Quantification of renal function, in: DiPiro, J., Talbert, R., Yee, G., Matzke, G., Wells, B., and Posey, L. **Pharmacotherapy: A Pathophysiologic Approach**, Sixth edition, (pp 753 - 769), United State of America: McGraw-Hill.

Dai, Y. Hebert, M. F. Isoherranen, N. Davis, C. L. Marsh, C. Shen, D.D. and Thummel, K. E. (2006), Effects of *CYP3A5* polymorphism on tacrolimus metabolic clearance in vitro. **Drug Metab Dispos**, 34, 836-847.

Daly, K., (2010), Pharmacogenetics and human genetic polymorphisms. **Biochem. J.**, 429, 435–449.

Eichelbaum, M. Fromm, M. and Schwab, M. (2004), Clinical aspects of the *MDR1* (*ABCB1*) Gene Polymorphism, **Ther Drug Monit**, 26 (2), 180-5

Elens, L. Capron, A. Kerckhove, VV. Lerut, J. Mourad, M. Lison, D. Wallemacq, P. and Haufroid, V. (2007), *1199G>A* and *2677G>T/A* polymorphisms of *ABCB1* independently affect tacrolimus concentration in hepatic tissue after liver transplantation. **Pharmacogenet Genomics**, 17 (10), 873-83

Fröhlich, M. Albermann, N. Sauer, A. Walter-Sack, I. Haefeli, W. E. and Weiss, J. (2004), *In vitro* and *ex vivo* evidence for modulation of P-glycoprotein activity by progestin. **Biochem Pharmacol**, 68 (12), 2409-16.

Fung, K. L. and Gottesman, M.M. (2009), A synonymous polymorphism in a common *MDR1* (*ABCB1*) haplotype shapes protein function. **Biochim Biophys Acta**, 1794(5), 860–871.

Gonzalez, T. Mucenic, T. Brenol, J. Xavier, R.M. Schiengold, M. and Chies, J. (2008), ABCB1 C1236T, G2677T/A and C3435T polymorphisms in systemic lupus erythematosus patients. **Braz J Med Biol Res**, 41, 769-772.

Goto, M. Masuda, S. Kiuchib, T Ogura, Y Oike, F. Okuda, M. Tanaka, K. and Inui, K. (2004), *CYP3A5*1*-carrying graft liver reduces the concentration/oral dose ratio of tacrolimus in recipients of living-donor liver transplantation. **Pharmacogenetics**, 14 (7), 471–478.

Hariharan, S. Christopher P. Barbara A. Sarah E. Matthew J. and Stablein, D. (2000), Improved graft survival after renal transplantation in the United States, 1988-1996. **N Engl J Med,** 342:605-12

Haufroid, V. Mourad, M. Van Kerckhove, V. Wawrzyniak, J. De Meyer, M. Eddour DC. Malaise, J. Lison, D. Squifflet, JP and Wallemacq P. (2004), The effect of *CYP3A5*

and *MDR1 (ABCB1)* polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. **Pharmacogenetics**, 14:147.

Hesselink, DA. van Schaik, RH. van der Heiden, IP. van der Werf, M. Gregoor, PJ. Lindemans, J. Weimar, W. and van Gelder, T. (2003), Genetic polymorphisms of the *CYP3A4, CYP3A5*, and *MDR-1* genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. **Clin Pharmacol Ther**, 74 (3), 245-54

Higgins, C. F. Callaghan, R. Linton, K. J. Rosenberg, M. F. and Ford, R. C. (1997), Structure of the multidrug resistance P-glycoprotein. **Cancer Biol.** 8, 135-142.

Hoffmeyer, S. Burk, O. and Von Richter, O. (2000), Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. **Proc Natl Acad Sci USA**, 97, 3473–3478.

Hosohata, K. Masuda, S. Yonezawa, A. Katsura, T. Oike, F. Ogura, Y. Takada, Y. Egawa, H. Uemoto S. and Inui, K. (2009), *MDR1* haplotypes conferring an increased expression of intestinal *CYP3A4* rather than *MDR1* in female living-donor liver transplant patients. **Pharm Res,** 26 (7), 1590-5

Innocenti, F. Deanna, L. Schuetz, K. E. Dolan, M. E. Ramirez, J. Relling, M. Chen, P. Das, S. Rosner, G. L. and Ratain, M. J. (2009), Comprehensive pharmacogenetic analysis of irinotecan neutropenia and pharmacokinetics. **J Clin Oncol**, 27 (16), 2604-1614.

Ishikawa, T. Onishi, Y. and Hirano, H. (2004), Pharmacogenomics of drug transporters: a new approach to functional analysis of the genetic polymorphisms of *ABCB1* (P-glycoprotein/ *MDR1*). **Biol Pharm Bull**, 27, 939–948.

Jain, B. Abu-Elmagd, K. Abdallah, H. Warty, V. Fung, J. Todo, S. Starzl, T. and Venkataramanan, R. (1993), Pharmacokinetics of FK506 in liver transplant recipients after continuous intravenous infusion, **J Clin Pharmacol**, 33; 606-611.

Johnson, H. and Schoder, R. (2005), Renal transplantation. In: DiPiro, J., Talbert, R., Yee, G., Matzke, G., Wells, B., and Posey, L. **Pharmacotherapy: A Pathophysiologic Approach**, (Sixth edition) (pp 843 - 866), McGraw-Hill.

Jonge, H. and Kuypers, D. (2008), Pharmacogenetics in solid organ transplantation: current status and future directions. **Transplant Rev,** 22, 6–20.

Kalble, T. Lucan, M. Nicita, G. Sells, R. Burgos F. J. and Wiesel, M. (2005), EAU guidelines on renal transplantation, **European Urology** 47, 156–166

Kapturczak, M. H. Meier-Kriesche, H. U. and Kaplan, B. (2004), Pharmacology of calcineurin antagonists, **Transplant Proc**, 36 (Suppl 2S), 25S-32S.

Kaya, P. Gunduz, U. Arpaci, F. Ural, A.U. and Guran, S. (2005), Identification of polymorphisms on the *MDR1* gene among Turkish population and their effects on multidrug resistance in acute leukemia patients. **American Journal of Hematology** 80:26–34.

Kerb, R. Aynacioglu, A.S. Brockmoller, J. Schlagenhaufer, R. Bauer, S. and Szekeres, T. (2001), The predictive value of MDR1, CYP2C9, and CYP2C19 polymorphisms for phenytoin plasma levels. **Pharmacogenomics J**; 1: 204-10.

Kim, H. C. Hwang, E. A. Han, S. Y. Park, S. B. Kim, H. T. and Cho, W.H. (2004), Primary immunosuppression with tacrolimus in kidney transplantation: three-year follow-up in a single center. **Transplant Proc,** 36, 2082–2083.

Kim, R. B. Fromm, M. F. and Wandel, C. (1998), The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. **J Clin Invest**, 101, 289–294.

Kim, R.B. Leake, B.F. and Choo, E.F. (2001), Identification of functionally variant MDR1 alleles among European Americans and African Americans, **Clin Pharmacol Ther**, 70, 189–199.

Kotrych, K. Sulikowski, T. Domanski, L. Bialecka, M. Gornik, W. and Drozdzik, M. (2007), Polymorphism in the P-glycoprotein drug transporter MDR1 gene in renal transplant patients treated with cyclosporine A in a Polish population, **Pharmacol Rep**, 59, 199-205

Kramer, K. Montagnino, G. Castillo, D. Margreiter, R. Sperschneider, H. Olbricht, C. Kruger, B. Ortuno, J. Kohler, H. Kunzendorf, U. H Stummvoll, H.K. Tabernero, J. Muhlbacher, F. Rivero, M. and Arias, M. (2005), Efficacy and safety of tacrolimus compared with cyclosporine A micro emulsion in renal transplantation. **Nephrol Dial Transplant**, 20: 968–973.

Leonard, G. D., Fojo, T., Bates, S. E. (2003), The role of ABC transporters in clinical practice. **Oncologist** 8, 411-424

Levey, A. S. Bosch, P. Lewis, J. B. Greene, T. Rogers, N. and Roth, D. (1999), A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. **Ann Intern Med***,* 130, 461-470.

Li, D. Gui, R. Li, J. Huang, Z. and Nie, X. (2006), Tacrolimus dosing in Chinese renal transplant patients is related to *MDR1* gene *C3435T* polymorphisms. **Transplant Proc**, 38, 2850–2852.

Li, D., Lu, W., Zhu, J.Y., Gao, J., Lou, T. Q. and Zhang, G.L. (2007), Population pharmacokinetics of tacrolimus and CYP3A5, MDR1 and IL-10 polymorphisms in adult liver transplant patients. **Clin Pharmacy & Ther**, 32, 505–515.

Macphee, I.A. Fredericks, S. Tai, T. Syrris, P. Carter, N.D. Johnston, A. Goldberg, L. and Holt, D.W. (2002), Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome P450 3A5 and P-glycoprotein correlate with dose requirement. **Transplantation**, 74 (11), 1486-9

Magee, C. and Pascual, M. (2004), Update in Renal Transplantation. **Arch Intern Med.,** 164:1373-1388.

Margreiter, R. (2002), Efficacy and safety of tacrolimus compared with cyclosporine micro emulsion in renal transplantation: A randomized multicentre study. **Lancet,** 359, 741-746

Marzolini, C. Paus, E. Buclin, T. and Kim, R.B. (2004), Polymorphisms in human *MDR1* (P-glycoprotein): Recent advances and clinical relevance. **Clin Pharmacother,** 75 (1), 13-33.

Matthew, H. (2004), Mechanisms of acute transplant rejection; **SCJMM**, 5:109-116.

Mathew, S. (2010), An overview on the allelic variant of *CYP2D6* genotype, **African Journal of Biotechnology,** Vol. 9 (54), 9096-9102.

Mendes, J. Martinho, A. Simoes, O. Mota, A. Breitenfeld, L. and Pais, L. (2009), Genetic polymorphisms in *CYP3A5* and *MDR1* genes and their correlations with plasma levels of tacrolimus and cyclosporine in renal transplant recipients. **Transplant Proc,** 41, 840–842.

Mickley, L.A. Lee, J.S. Weng, Z. Zhan, Z. Alvarez, M. Wilson, W. Bates, S. E. and Fojo, T. (1998), Genetic polymorphism in MDR-1: a tool for examining allelic expression in normal cells, unselected and drug-selected cell lines, and human tumors. **Blood**, 91, 1749—1756

Mourad, M. Mourad, G. Wallemacq, P. Garrigue, V. Van Bellingen, C. Van Kerckhove, V. De Meyer, M. Malaise, J. Eddour, D.C. Lison, D. Squifflet, J.P. and Haufroid, V. (2005), Sirolimus and tacrolimus trough concentrations and dose requirements after kidney transplantation in relation to *CYP3A5* and *MDR1* polymorphisms and steroids. **Transplantation**; 80:977-84.

Mourad, M. Wallemacq, P. De Meyer, M. Malaise, J. De Pauw, L. Eddour, D. Goffin, E. Lerut, J. and Haufroid, V. (2008), Biotransformation enzymes and drug transporters pharmacogenetics in relation to immunosuppressive drugs: impact on pharmacokinetics and clinical outcome. **Transplantation,** 85 (7S), S19-24

Murray, J. Merril, J. and Harrison, J. (2001), Renal homotransplantations in identical twins. **J Am Soc Nephrol**, 201, 201-204.

Nankivell, B.J. Chapman, J. Bonovas, G. and Gruenewald, S. (2004), Oral cyclosporine but not tacrolimus reduces renal transplant blood flow. **Transplantation**, 77 (9), 1457–9

Pechandová, K. Buzková, H. Slanar, O. and Perlík, F. (2006), Polymorphisms of the *MDR1* gene in the Czech population, **Folia Biol (Praha),** 52 (6), 184-9

Pirsch, J.D. Miller, J. Deierhoi, M.H. Vincenti, F. Filo R.S. (1997), A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. **Transplantation**, 15; 63(7):977-83.

Pocket guideline of Prograf® recommendations from Hikma, manufacturing company of Prograf® (Tacrlolimus) in Jordan.

Provenzani, A. Notarbartolo, M. Labbozzetta, M. Poma, P. Biondi, F. Sanguedolce, R. Vizzini, G. Palazzo, U. Polidori, P. Triolo, F. Gridelli, B. and D'Alessandro, N. (2009), The effect of CYP3A5 and ABCB1 single nucleotide polymorphisms on tacrolimus dose requirements in Caucasian liver transplant patients. **Ann Transplant.**, 14(1):23-31.

Rosenberg, M.F. Callaghan, R. Ford, R.C. and Higgins, C.F. (1997), Structure of the multidrug resistance P-glycoprotein to 2.5 nm resolution determined by electron microscopy and image analysis. **J. Biol. Chem.**, 272, 10685-10694

Roy, J.N. Barama, A. Poirier, C. Vinet, B. and Roger, M. (2006), *CYP34, CYP3A5* and *MDR-1* genetic influence on tacrolimus pharmacokinetics in renal transplant recipients, **Pharmacogenet Genomics,** 16 (9), 659-65.

Said, R. (1999), Renal transplantation in Jordan, **Saudi J Kidney Dis Transplant**, 10(1): 64-65.

Sakaeda, T. Nakamura, T. and Okumura, K. (2002), *MDR1* genotype-related pharmacokinetics and pharmacodynamics, **Biol Pharm Bull,** 25 (11), 1391—1400

Sakurai, A. Onishi, Y. Hirano, H. Seigneuret, M. Obanayama, K. Kim, G. Liew, E.L. Sakaeda, T. Yoshiura, K. Niikawa, N. Sakurai, M. Ishikawa, T. (2007), Quantitative structure--activity relationship analysis and molecular dynamics simulation to functionally validate non-synonymous polymorphisms of human ABC transporter ABCB1 (P -glycoprotein/MDR1), **Biochemistry**; 46(26):7678-93.

Schaeffeler, E. Eichelbaum, M. Brinkmann, U. Penger, A. Asante-Poku, S. Zanger, U. M. Schwab, M. (2001), Frequency of C3435T polymorphism of MDR1 gene in African people, **Lancet** 358, 383-384.

Scott, L.J. McKeage, K. Keam, S.J. Plosker, G.L. (2003), Tacrolimus: a further update of its use in the management of organ transplantation. **Drugs**; 63(12):1247-1297.

Sommerer, C. Hergesell, O. Nahm, A.M. Schwenger, V. Waldherr, R. Andrassy, K. and Zeier, M. (2002), Cyclosporine A toxicity of the renal allograft--a late complication and potentially reversible. **Nephron**; 92(2):339-45.

Staatz, C.E. Tett, S.E. (2004), Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation, **Clin Pharmacokinet**, 43:623-53.

Sun, Q. Liu, Z. Yin, G. Chen, H. Ji, S. and Li, L. (2006), Tacrolimus combined with mycophenolate mofetil can effectively reverse C4d-positive steroid-resistant acute rejection in Chinese renal allograft recipients. **Nephrol Diol Transplant,** 21, 510-517.

Tada, H. Tsuchiya, N. Satoh, S. Kagaya, H. Li, Z. Sato, K. Miura, M. Suzuki, T. Kato, T. and Habuchi, T. (2005), Impact of *CYP3A5* and *MDR1(ABCB1) C3435T* polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. **Transplant Proc**, 37 (4), 1730-2

Talberard, E. and Dupuis, R. (2005), Solid organ transplantation, In: Koda-Kimble, M., Young, L., Kradjan, W., Guglielmo, B. **Applied Therapeutics: The Clinical Use of Drugs**, (Seventh edition) (pp 33-1 – 33-50), United States of America: Lippincott Williams and Wilkins.

Tanabe, M. Ieiri, I. and Nagata, N. (2001), Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. **J Pharmacol Exp Ther**, 297 (3), 1137-43.

Taylor, A. Watson, C. and Bradley, J. (2005), Immunosuppressive agents in solid organ transplantation: Mechanisms of action and therapeutic efficacy. **Crit Rev Oncol Hematol,** 1, 23-46.

Thervet, E. Anglicheau, D. Legendre, C. and Beaune, P. (2008), Role of pharmacogenetics of immunosuppressive drugs in organ transplantation, **Ther Drug Monit,** 30 (2), 143-50

Tsuchiya, N. Satoh, S. Tada, H. Li, Z. Ohyama, C. Sato, K. Suzuki, T. Habuchi, T. and Kato, T. (2004), Influence of *CYP3A5* and *MDR1 (ABCB1)* polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. **Transplantation**, 78 (8), 1182-1187.

Ulemat, M. (2010), **Impact of genetic polymorphism of *ABCB1 (MDR1) G2677T/A* in kidney donors on tacrolimus level in Jordanian kidney transplant recipients**, Master Thesis, University of Jordan, Amman, Jordan

Undre, N.A. van Hooff, J. Christiaans, M. Vanrenterghem, Y. Donck, J. Heeman, U. Kohnle, M. Zanker, B. Land, W. Morales, J.M. Andres, A. Schafer, A. and Stevenson P. (1999), Low systemic exposure to tacrolimus correlates with acute rejection. **Transplant Proc**; 31:296-8.

Venkataramanan, R. Swaminathan, A. Prasad, T. Jain, A. Zuckerman, S. Warty, V. McMichae, J. Lever, J. Burckart, G. and Starz, T. (1995), Clinical pharmacokinetics of tacrolimus.**Clin Pharmacokinet,** 29, 404–30.

Verhulst, A. Sayer, R. De Broe, ME. D'Haese, P. and Brown, C. (2008), Human proximal tubular epithelium actively secretes but does not retain rosuvastatin. **Mol Pharmacol**, 74 (4), 1084–1091.

Vicari-Christensen, M. Repper, S. Basile, S. Young, D. (2009), Tacrolimus: review of pharmacokinetics, pharmacodynamics, and pharmacogenetics to facilitate practitioners' understanding and offer strategies for educating patients and promoting adherence. **Transplantation**, 19 (3)

Wallemacq, P. Armstrong, V.W. Brunet, M. Haufroid, V. Holt, D.W. Johnston, A. Kuypers, D. Le Meur, Y. Marquet, P. Oellerich, M. Thervet, E. Toenshoff, B. Undre, N. Weber, L.T. Westley, I.S. Mourad, M. (2009), Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference, **Ther Drug Monit**.;31(2):139-52.

Wang, W. Zhang, X.D. Ma, L.L. Lü, Y.P. Hu, X.P. Zhang, P. Wang, Y. and Guan, D.L. (2005), Relationship between *MDR1* gene polymorphism and blood concentration of tacrolimus in renal transplant patients, **Zhonghua Yi Xue Za Zhi**, 85 (46), 3277-81.

Wavamunno, M. and Chapman, J. (2008), Individualization of immunosuppression: concepts and rationale. **Curr OpinOrgan Transplant**, 13, 604–608.

Williams, R. Neuhaus, P. Bismuth, H. McMaster, P. Pichlmayr, R. Calne, R. Otto, G. Groth C. (1996), Two-year data from the European multicentre tacrolimus (FK506) liver study. **Transpl Int**, 9 Suppl 1:S144-50.

Yan-Hong, L. Yong-Hua, W. Yan, L. and Ling, Y. (2005), *MDRl* Gene Polymorphisrns and Clinical Relevance. **Acta Genetica Sinica**, 33, 93-104.

Yano, I. (2008), Pharmacodynamic monitoring of Calcineurin inhibitors. **Drug Metab. Pharmacokinet**. 23 (3): 150–157.

Zheng, H. Webber, S. Zeevi, A. Schuetz, E. Zhang, J. Bowman, P. Boyle, G. Law, Y. Miller, S. Lamba, J. and Burckart, GJ. (2003), Tacrolimus dosing in pediatric heart

transplant patients is related to *CYP3A5* and *MDR1* gene polymorphisms. Am J **Transplant**, 3 (4), 477-83.

Zhou, S. F. (2008), Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition**. Xenobiotica**, 38 (7–8), 802–832.

Appendix I
Ethical Approval

بسم الله الرحمن الرحيم

G. H. Q. Jordan Armed Forces
DIRECTORATE
ROYAL MEDICAL SERVICES
Director of Professional Training
and Human Resources Development
Amman – Jordan

القيادة العامة للقوات المسلحة الأردنية
مديريـــــــــة
الخدمـــــات الطبية الملكيـــة
مديرية التأهيل الفني وتنمية القوى البشرية
عمان – الأردن

الرقـم : ت ف ٣/١ / ١٩٠٥٤

التاريخ : رمضـــــان ١٤٣١
١٥ آب ٢٠١٠

الجامعـــــــة الاردنيـــــــــة

الموضوع : الدراسات

تحيه طيبه وبعد ....

الاشارة: كتابكم رقم ٣٣٩٤/١/٨ تاريخ ٢٠١٠/٦/١٤

* اوصت لجنة البحوث والدراسات السريرية والدوائية واخلاقيات المهنة باجتماعها الذي عقد بتاريخ ٢٠١٠/٨/١٧ بالموافقة على ماجاء بكتابكم الاشارة اعلاه بخصوص اجراء دراسة من قبل طالب الماجستير النقيب الصيدلاني احمد علي سليم مساعده حول اثر التباين الجيني لـــ ABCB1 (MDRI) 2677 G > T- A في المتبرعون بالكلى على مستوى التاكروليمس في متلقي زرع الكلى الاردنيين خلال الفترة المبكرة ما بعد الزرع ٠

واقبلوا الاحترام ،،،،،

عـ/ اللواء الطبيب
مدير عام الخدمات الطبيه الملكيــــــة

نسخه الى :

- مستشفى الحسين
- مدير التأهيل الفني وتنمية القوى البشرية
- رئيس شعبة امن الخدمات الطبية
- مدير مكتب عطوفة المدير العام
- سكرتير لجنة اخلاقيات المهنة
- الاضبارة العامة ١/٣/أ /
- التداول

FAX: 962-6-5347485 TEL: 5804804 EXT: 4341/ 4373 P.O. BOX: 122 Dahyat Al-Amir Rashid, Amman 11831 Jordan
فاكس : ٥٣٤٧٤٨٥ ـ ٦ ـ ٩٦٢ ، تلفون: ٥٨٠٤٨٠٤ فرعي ٤٣٧٣/٤٣٤١/٤٣٤٢ ، ص.ب ١٢٢ ضاحية الأمير راشد، عمان ١١٨٣١ الأردن

Appendix II

Consent forms

For both donors & recipients

Consent form

Aug 2010

Title of study: Impact of genetic polymorphism of ABCB1 (MDR1) 2677G>T –A in kidney donors on tacrolimus level in Jordanian kidney transplant recipients during the early post transplantation period.

Sponsor: University of Jordan.

Investigator:
Student name: Ahmad Ali Saleem Masadeh.
Department of Biopharmaceutics and Clinical Pharmacy/ Faculty of Pharmacy/ University of Jordan

Subject Name: - ……………………………………………………………………

This consent form may contain words that you do not understand. Please ask the study investigator to explain any words or information that you do not clearly understand.

The Nature and Purpose of This Study
You are being asked to voluntarily take part in a research study which will involve only once drawing of 3 ml of blood for genetic study from your vein at the same time your blood sample for drug serum concentration will be obtained. The medications that you are taking will remain unchanged.
The purpose of this study is to detect the relation of your genetic characteristics to the dosage requirements of your medication (Tacrolimus).

Duration
You will be enrolled in a study only for one day to obtain a blood sample.
Enough subjects will be enrolled in order to study at least 40 blood samples.

Explanation of Experimental Procedures to be followed
If you do volunteer, it will be necessary to draw about 3 ml of venous blood at the same time as your blood drawing for Tacrolimus level.
You will need to inform the investigator about your medical history and any medications you are taking. You must not participate if you are a female who is pregnant.

Possible Risks/Discomforts
None

Benefits
It is possible that following completion of this study better treatment program will be applied to yourself or future subjects with renal transplants.

Payment
You will not be paid for the participation of this study. Neither will you be expected to pay for any study-related expenses.
Voluntary Participation
You understand that participation in this study is voluntary. You understand that a decision not to participate in this study will not influence the availability of future medical care.

You understand that the investigator of the study can remove you from the study without your consent using his/her judgment.

Contact or Questions

If you have any questions during the study, contact:

Dr. Ibrahim Smadi, King Husain Hospital, Tel: 0777742667

If you have any questions regarding your rights as a subject, you may contact: 5840840, Institutional Review Board/Institutional Ethical Committee (IRB/IEC)

Who Will Know That I am in this study?

Your records obtained while you are in this study will remain strictly confidential at all times. However, they will need to be made available to others working on sponsor's behalf, the Institutional Review Board and medicine regulatory authority [e.g. the Food and Drug Administration (FDA)].

By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it. The information disclosed will remain confidential.

Consent to Participate in This Study

I have read, or had read to me in my first language, the above information. The content and meaning of this information has been explained to me. I have had an opportunity to ask questions about this study and this consent form and have received answers that fully satisfied those questions. I have read all pages of this informed consent form, and I understand the consent form risks described. I freely and voluntarily consent and offer to take part in this study. By signing the consent form, I certify that all information I have given. Including my medical history is true and correct to the best of my knowledge.

I understand that I will receive a copy of this consent form.

I authorize the release of my medical records to the sponsor and the FDA.

By signing this consent form I have not waived to the legal rights which I otherwise would have as a participant of a study.

………………………………………………………..

Participant's Signature Date

(Or Subject's Legally Authorized Representative)

………………………………………………………..

Person Obtaining Consent Date

…………………………………

Witness (if participant cannot read) Date

استمارة الإقرار بالعلم والموافقة

اسم الدراسة: اثر التباين الجيني ل *ABCB1 (MDR1) 2677G>T* –A في المتبرعون بالكلى على مستوى التاكروليمس في متلقي زرع الكلى الاردنيين خلال الفترة المبكرة ما بعد الزرع.

راعي الدراسة: الجامعة الأردنية

الباحث:
الطالب: احمد علي سليم مساعده
قسم الصيدله السريريه والحيويه/ كلية الصيدله/ الجامعة الأردنيـــة

اسم الشخص المشارك..

قد تحتوي استمارة الإقرار والموافقة هذه على كلمات قد لا تفهمها. من فضلك اسأل باحث الدراسة لشرح أي كلمات أو معلومات لا تفهمها بوضوح.

طبيعة وغرض هذه الدراسة:
نود منك المشاركة بدور في دراسة بحثية ستستلزم سحب 3 ملم من الدم في نفس الوقت الذي سوف يسحب منك عينة دمك لقياس مستوى الأدوية التي تأخذها ولمره واحدة. هذا مع العلم أن الأدوية التي تأخذها لن تتغير.
الغرض من هذه الدراسة هو اكتشاف أي علاقة بين خواصك الجينية والجرعة المناسبة من أدويتك (التاكروليمس) .

المـــدة:
ستقيد في دراسة لمدة يوم واحد فقط للحصول على عينة الدم.
عدد الأشخاص الكلي المشاركين في هذه الدراسة هو (40) شخص على الاقل.

تفسير الإجراءات المخبرية المتبعة:
إذا تطوعت سيكون ضرورياً أن يتم سحب حوالي 3 ملم من الدم في نفس الوقت لقياس مستوى جرعة التاكروليمس. سوف تحتاج لإعلام الباحث عن تاريخك المرضي و عن الأدوية التي تتناولها.
لا يجب أن تشاركي إذا كنت تعلمين أو تشكين بأنك حامل.

الأخطار والمشقات المحتملة من المشاركة:
لا شيء

فوائد الدراسة:
من الممكن بعد إكمال هذه الدراسة توفر نظم علاجية أفضل لك أو لأشخاص مثل حالتك في المستقبل.

النفقات المالية:
سوف لن يتم دفع أي مبلغ من المال لك مقابل مشاركتك الطوعية في هذه الدراسة ولن يطلب منك المساهمة في أي من المصاريف المالية.

المشاركة الطوعية:
أنت تفهم أن المشاركة في هذه الدراسة طوعية. وأنت تفهم أن قرارك بعدم المشاركة في هذه الدراسة لن يؤثر على توفر الرعاية الطبية المستقبلية لك.
أنت تفهم أيضاً أن الباحث يمكن أن يطلب منك الانسحاب من الدراسة بدون موافقتك المسبقة وفقاً لتقديراته.

<u>للاتصال أو الاستفسار:</u>
إذا كان لديك أي استفسار أثناء الدراسة، يرجى الاتصال بــ :
د. ابراهيم صمادي, مستشفى الحسين (المدينة الطبية) : استشاري أمراض باطنية وكلى,
تلفون0777742667
إذا كان لديك أي استفسار بخصوص حقوقك القانونية يرجى الاتصال بــ 5804804
مجلس المراجعة الخاصة بالمؤسسة / اللجنة الأخلاقية.

<u>من سيعرف أنني مشارك في هذه الدراسة؟</u>
المعلومات المستقاة من سجلاتك الطبية أثناء هذه الدراسة ستبقى سرية. قد يحتاج مجلس المراجعة الخاصة بالمؤسسة / اللجنة الأخلاقية أو أي من السلطات القانونية (مؤسسة الغذاء والدواء) للإطلاع على بعض هذه المعلومات.
بتوقيعك بالموافقة على هذه الاستمارة أنت توافق ضمنياً على الاطلاع على المعلومات في سجلك الطبي لغرض هذه الدراسة أو أي بحوث قادمة لها علاقة بالدراسة الحالية.

<u>الموافقة على المشاركة في هذه الدراسة:</u>

أقر أنه:
* قد قرأت أو قُرئ لي بلغتي الأم المعلومات السابقة.
* محتوى ومعنى هذه المعلومات قد شُرح لي.
* كان لدي الفرصة للاستفسار عن هذه الدراسة واستمارة الإقرار هذه وقد تلقيت الإجابات التي أرضتني تماما.
* قد قرأت كل صفحات استمارة الإقرار بالعلم هذه وأقر أنني أفهم استمارة الإقرار والأخطار الموصوفة.

أنا بكامل حريتي واختياري:
*أوافق، وأرفض المشاركة في هذه الدراسة.
*بتوقيع استمارة الموافقة، أشهد أن كل المعلومات التي أعطيتها، متضمنة تاريخي الطبي، حقيقية وصحيحة على حد علمي.
*أفهم أنني سأتلقى نسخة عن استمارة الإقرار بالعلم
*أصرح بتحرير سجلاتي الطبية إلى راعي الدراسة ولمؤسسة الغذاء والدواء
*بتوقيعي على استمارة الإقرار هذه لم أتخلَ عن أي من حقوقي القانونية

توقيع المشارك (أو من هو مفوض عنه قانونياً)	التاريخ
....	....
توقيع طالب الإقرار	التاريخ
....	....
توقيع الشاهد	التاريخ
....	....

Appendix III

Data collection form

CRF No:
Date of data collection:

Administrative and Demographic Information

Participant Name:	Participant ID:
Gender: ☐ male ☐ female	Date of Birth: Age:
Height (cm):	MD (consultant):
Weight (kg):	File No:
BMI: IBW:	Admission Date:
Underweight-Normal-Overweight-Obese-morbid Obesity	Room No:
General Health on a Scale of 10 (1 very bad-10 very good)	Discharge Date:
Smoking History ☐ smoker ☐ ex-smoker ☐ never smoked	Occupation:
Ethnic Origin ☐ black ☐ Caucasian ☐ others (specify)	Phone #:
Nationality:	Martial state:
Family history:	Allergy:
Lifestyle (diet, exercise,alcohol,caffeine):	Address:

Transplant History			
Transplant number		HLA mismatches (A,B,DR, median/range)	
Last Transplant		HLA DR mismatches 0/1/2	
Donor's age at the time of transplantation		Donor's gender	
Donor's relation to recipient		Panel reactive antibody >50% (peak/at transplantation)	
Hospital stay (days)		Dialysis duration before transplantation (months)	

Primary kidney disease:	Past medical History / surgery	Acute and chronic medical problems (class/duration)
☐ GM (Glomerulnerphritis)	1…………………………………	1………………………………….
☐ Chronic pyelonephritis	2………………………………….	2………………………………….
☐ Ig A nephropathy	3………………………………….	3…………………………………..
☐ Diabetic nephropathy	4………………………………….	4…………………………………..
☐ Hypertensive nephropathy	5…………………………………..	5…………………………………..
☐ Polycystic Kidney disease	6…………………………………..	6…………………………………..
☐ Other (specify)	7…………………………………..	7…………………………………..
☐ unknown		

Current Medication						
Indication	Drug name Brand	Drug name Generic	Strength	Frequency	Route	Duration

Vital Signs and Lab Data (Intial and Follow Up)										
Date	**Last visit**	**- 1 mo**	**- 2 mo**	**- 3 mo**	**- 4 mo**	**- 5 mo**	**- 6 mo**	**Immediate post Tx**	**Baseline (before tr)**	**Reference**
Wt										
Systolic BP										
Diastolic BP										
Pulse										
Resp.Rate										
Sr.Cr										
Cl.Cr										
BUN										
BUN/Cl										
Uric Acid										
Na										
K										
Cl										
Ca										
PO4										
Mg										
Homocystein										
ALK.P										
ALT										
AST										
GGT										
Glucose										
PT										
a.PTT										
Bil.D										
Bil.T										
Albumin										
T.Protein										
PLT										
RBCs										
WBCs										
Hb										
Hct										
MCV										
MCHC										
MCH										
T.Cholestrol										
LDL-C										
HDL-C										
TG										
ESR										

Date	Tacrolimus level	Tacrolimus dose	Action

History of rejection:

1. **Date**____
2. **Type**____
3. **Complains**____________________________________
4. **Physicalexam**__________________________________
5. **Results of biopsy**______________________________
6. **Lab data (Cr, BUN, fluid ins/outs…)** ______________________
7. **Treatment used**________________________________

Tacrolimus neurological, dermatological and miscellaneous ADRs

Characteristic	Date & Duration
Neurological ADRs	
Tremor	
Seizures	
Headache	
paresthesias	
Sleep disturbances	
Asthenia	
Dizziness	
Dermatological and cosmetic ADRs	
Pruritis & rash	
Alopecia	
Hirsuitism	
Gum hyperptophy	
Warts	
Hematologic ADRs	
Anemia (N=136)	
Thrombocytopenia (N=93)	
Leucopenia (N=136)	
Other ADRs	
Diarrhea	
Recurrent UTI	
Nausea & vomiting	
Infection	
Malignancies	
Hypertension	
hyperglycemia	
Dyslipidemia	
CVD	

Appendix IV

Extraction of genomic DNA from blood samples

1. 900 µl of cell lysis solution was added to a sterile 1.5 ml micro-centrifuge tube.
2. The tube of blood was gently rocked until thoroughly mixed; then 300 µl of blood was transferred to the tube containing the cell lysis solution, and the tube was inverted 5 to 6 times to mix.
3. The mixture was incubated for 10 minutes at room temperature (inverted 3 times during the incubation) to lyse the red blood cells. The mixture then was centrifuged at 15000 x *g* for 20 seconds at room temperature.
4. Supernatant was removed and discarded without disturbing the visible white pellet.
5. Approximately 10 to 20 µl of residual liquid remained in the 1.5 ml tube. Then the tube was vortexed vigorously until the white blood cells were resuspended (20 seconds).
6. 300 µl of nuclei lysis solution was added to the tube containing the resuspended cells; then the solution was pipetted 5 to 6 times to lyse the white blood cells.
7. RNAse solution (1.5 µl) was added to the nuclear lysate and the sample was mixed by inverting the tube 10 times.
8. The mixture was incubated at 37°C for 15 minutes, and then cooled to room temperature.

9. The protein precipitation solution (100 µl µl) was added to the nuclear lysate and vortexed vigorously for 20 seconds. Small protein clumps were visible after vortexing.
10. The mixture was centrifuged at 15000 x *g* for 3 minutes at room temperature. A dark brown protein pellet was visible at the bottom of micro-centrifuge tube.
11. To precipitate the DNA, the supernatant was transferred to a clean 1.5 ml micro-centrifuge tube containing 300 µl of room temperature isopropanol.
12. The tube was inverted several times until white threads of DNA were observed in the solution, and then the mixture was centrifuged at 15000 *xg* for 1 minute at room temperature. The DNA was visible as a small white pellet.
13. Then the supernatant was decanted and 300 µl of room temperature 70% ethanol was added to the DNA.
14. Tube was gently inverted several times to wash the DNA pellet and the sides of the micro-centrifuge tube, then the mixture was centrifuged at 15000 x *g* for 1 minute at room temperature. The DNA was visible as a small white pellet.
15. Ethanol was carefully aspirated by using a drawn Pasteur pipette connected to evacuated pump. After that the DNA pellet was air-dried for 15 minutes.
16. DNA was rehydrated by addition of 100 µl of DNA rehydration solution.
17. Finally, DNA was stored at 4 °C until further analysis.

Appendix V:

Preparation of solutions and reagents

0.5 M EDTA solution

Add 186.1 gm of EDTA.2H2O to 800 ml H2O. Stir vigorously on a magnetic stirrer. Adjust pH to 8.0 with NaOH.

Tris. Borate (TBE) Buffer (10X)

1. 100 ml of ddH2O in 1000ml volumetric flask

2. 21.8 gm Tris base

3. 11.12 gm boric acid

4. 1.48 EDTA (pH = 8.0).

5. Adjust volume to 200 ml with ddH2O.

Store in glass bottles at room temperature and discard any batches that develop a precipitate.

Tris.Cl Solution (1 M)

1. Dissolve 121.1 gm Tris base in 800 ml of H2O, adjust pH to 8 by adding 42 ml Concentrated HCl

2. Allow the solution to cool to room temperature before making final adjustments to pH Adjust the volume of the solution to 1 liter with H2O.

3. Dispense in aliquots and sterilize by autoclaving.

Tris. EDTA Buffer (pH = 8)

1. 10 mM Tris.Cl (pH = 8)

2. 1 mM EDTA (pH = 8)

Appendix VI
Hardy-Weinberg Equilibrium Calculation

Number of Patients with GG (2 alleles of G) = 28

Number of Patients with TT (2 alleles of T) = 5

Number of Patients with GT (1 alleles each) = 20

So;

Number of G alleles is 28*2+20*1 = 76

Number of T alleles is 5*2+20*1 = 30

G allele frequency = 76/106

= 0.72

T allele frequency = 30/106

= 0.283

According to the equation:

$G^2+2GT+T^2=1$ ……………………………………equation 1

To solve equation 1:

$(0.72)^2 + 2(0.72*0.28) + (0.28)^2 = 1$

Number of patients expected in each genotype group according to H-W is;

$G^2 = 0.514$………………

Number of patients expected for GG in sample equal 53 is 0.514*53= 27

$T^2= 0.080$…………………..

Number of patients expected for TT in sample equal 53 is 0.080*53= 4

2GT=0.40752………………

Number of patients expected for GT in sample equal 53 is 2*53*0.72*0.28= 22

أثر التباين الجيني ل *ABCB1 (MDR1) 2677G>T* –A في المتبرعين بالكلى على مستوى التاكروليمس في متلقي زراعة الكلى الاردنيين خلال الفترة المبكرة بعد الزراعة.

اعداد

أحمد علي سليم مساعدة

المشرف

د. نائله بولاتوفا

المشرف المشارك

د. المعتصم يوسف

ملخص الدراسة

يستخدم التاكروليمس على نطاق واسع لمنع الرفض المناعي في مرضى زراعة الكلى، حيث يتميز التاكروليمس بضيق المؤشر العلاجي وتباين ملحوظ داخل و بين الافراد، لذلك مراقبة مستوى التاكروليمس في الدم و تحقيق مستويات الحد الادنى من العلاج تعتبر ضرورة لتحقيق التأثير الأمثل للحد من الرفض المناعي وسمية العلاج وذات أهمية قصوى خلال الفترة المبكرة ما بعد الزراعة. بعض تعدد الأشكال الوراثية في البروتينات الناقلة مثل ب جلايكو بروتين المشفرة بواسطة جينات *ABCB1)MDR1)* في الجهات المانحة و / أو المتلقين تظهر كمحددات هامة للحرائك الدوائية للتاكروليمس. فإلى حد علمنا، لا توجد دراسات سابقة قيمت أثر تعدد الأشكال الوراثية *ABCB1 (MDR1) 2677G>T –A* في المتبرعين بالكلى على مستوى التاكروليمس في متلقي الكلى الاردنيين خلال الفترة المبكرة ما بعد الزراعة.

كان الهدف من هذه الدراسة هو تحديد أثر التباين الجيني في متبرعي الكلى على الجرعة التي يحتاجها الجسم، مستوى العلاج الفعال، و مستوى العلاج الفعال بالنسبة للجرعة لزارعي الكلى الاردنيين خلال الفترة المبكرة ما بعد الزراعة. حيث تم تحديد الطبيعة الجينية للمتبرعين وعددهم (53) متبرع للجين المذكور باستخدام PCR-RFLP. جرعات التاكروليمس ومستوياته في دم زارعي الكلى تم مقارنتها بناءا على الطبيعة الجينية للمتبرعين *ABCB1 MDR1* عند الموقع *G26677T/A*. تم الحصول على الخصائص الديموغرافية والبيانات السريرية للمرضى الزارعين من ملفاتهم والمقابلات المباشرة معهم عند دخول المسشفى و متابعتهم لمدة 6 أشهر من بعد الزراعة.

نتائج الدراسة الحالية تكشف عن أن الطبيعة الجينية تتوزع حسب الاعداد والنسب التالية : من 53 متبرع كان هناك 28 (%52.8) *GG*، 20 (37.7%) *GT* ، 5 (%9.4) *TT* على الترتيب من المتبرعين يحملون الطبيعة الجينية. مستوى علاج التاكروليمس الفعال عند الزارعين من متبرعين يحملون على الاقل نسخة واحدة من الأليلة *T (GT* او *TT)* أظهر فرق غير ملحوظ مقارنة مع مستوى العلاج في في الطبيعة الجينية (*GG*) أثناء الشهور الستة الأولى بعد زراعة الكلية. لم يكن هناك اي فروقات ملحوظة بين المجموعتين بخصوص اعمار الزارعين، اوزانهم، معامل وزن الجسم، مستوى الزُلال، خضاب الدم، تردد جنس الزارع، جنس المتبرع، التوافق بين جنس الزارع والمتبرع، مستخدمي مثبطات قنوات الكالسيوم، وجرعة الكورتيكوستيرويد بين المجموعتين. حتى الآن، نتائج الدراسات تبقى جدالية و يجب الاخذ بعين الإعتبار للعديد من العوامل الأخرى.